or coadministration of anti-inflammatory drugs such as steroids [147–150]. Lieber et al. [145] obtained persistent Ad-delivered hAAT [human alpha (1)-antitrypsin] expression in bcl-2 transgenic mice for longer than 3 months by coadministrating an Ad vector expressing an IκBα supersuppressor to unravel the role of virus-induced NF κB activation [151,152]. They found that to confer vector persistence, simultaneous expression of bcl-2, an antiapoptotic protein [153], was required to block virus-induced apoptosis, while NF κB protection was inactivated by the IκBα supersuppressor. Kolb et al. [147] investigated the use of topical corticosteroids in improving gene expression after repeated injection of Ad vectors into mouse lungs. They showed that budesonide given around exposure to Ad to the lung significantly helped maintain high levels of the expressed transgene protein in bronchoalveolar lavage fluid after as many as four consecutive injections of virus at 2-week intervals. Furthermore, they observed that the improved transgene expression in budesonide-treated animals was associated with a reduction, but not prevention of neutralizing antiviral antibodies.

Another approach is the use of immunosuppressive drugs to inhibit cell-mediated immune functions, just as they are used in clinical trials for organ transplantation. The central role of CD4+ T cells in the activation of cellular and humoral immune response has focused immunosuppressive strategies towards blockade of costimulatory molecules. Recombinant murine CTLA4Ig (an inhibitor of the CD28/B7 pathway) and the anti-CD40 ligand antibody block costimulatory interactions between T cells and antigen presenting cells. Blocking of costimulatory signals has been shown to inhibit T-cell activation in several animal models of Ad vector gene therapy. Kay et al. [154] observed that when the anti-CD40 ligand and recombinant murine CTLA4Ig were coadministered around the time of primary Ad vector administration, Ad-mediated gene expression was maintained up to 1 year in mouse livers, and persistent secondary Ad-mediated gene expression lasted for at least 200-300 days even after the immunosuppressive effects of these agents were no longer present. However, neither agent alone allowed transduction after secondary vector administration. Jooss et al. [155] described a strategy that aimed to inhibit CD4+ T cell activation by transiently administering CTLA4Ig at the time an E1-deleted Ad vector is administered to the liver or lung. In the lung, CTLA4Ig treatment significantly blocked the formation of neutralizing antibodies, allowing efficient readministration of the vector, whereas transgene expression was only moderately prolonged. In contrast, CTLA4Ig did not suppress neutralizing antibody formation in the context of liver gene therapy, but resulted in more stable gene expression. These observations suggest that it may be possible to obtain persistence as well as secondary Ad-mediated gene transfer with transient inhibition of the CD28/B7 pathway at the time of virus instillation, especially in a local route. However, immune intervention in systemic Ad administration seems to be much complex. Moreover, this immunomodulation might compromise the host immune response. Thus, the clinical utility is uncertain.

4.5. Optimization of in-cis acting elements

As discussed in the previous section, the immunogenic toxicities of Ad vectors can lead to clearance of the transduced cells and only transient transgene expression. The severity of the immunogenic toxicities is intimately connected with vector dose. However, to achieve anatomically detectable and physiologically relevant levels of transgene expression, the number of Ad particles to be injected is always too large to avoid severe immunological side effects [156,157]. One simple but practical way to alleviate this kind of immunogenic toxicities is to improve the expression efficiency of each viral particle unit so that the number of Ad particles to be injected can be decreased.

Transgene expression can be modulated at both the transcriptional and post-transcriptional levels. We systematically investigated the ability of transcriptional regulatory elements [promoter, enhancer, intron and poly(A) sequences] and post-transcriptional regulatory elements to maximize the transgene expression efficiency from each Ad vector unit [158]. Our optimized Ad vector, Ad-WCMVL2, with an expression cassette containing the human CMV promoter/enhancer, intron A, the luciferase gene, the Woodchuck hepatitis virus post-transcriptional regulation element (WPRE), and bovine growth hormone (BGH) poly (A), showed more than 700-fold luciferase expression in mouse liver than the Ad vector, Ad-

CMVL1, with an expression cassette containing only the human CMV promoter/enhancer, the luciferase gene and BGH poly (A), when they were intravenously administered into the mouse [158]. It may be deduced that to express transgenes at a therapeutic level, the in-cis acting elements of our optimized Ad vector, Ad-WCMVL2, may decrease, by several orders of magnitude, the number of viral particles that must be injected as compared with Ad-CMVL1 with the expression cassette containing only the CMV promoter/enhancer and the BGH poly(A) as in-cis acting elements which are considered to be one of the most strongest in-cis acting element combination and are widely used in clinical trials and gene function studies [159]. It also means that immunogenic toxicities induced by Ad particles could be greatly alleviated by using the in-cis acting elements of Ad-WCMVL2 to construct therapeutic Ad vectors. Gerdes et al. [160] demonstrated that the murine CMV promoter was much stronger than the human CMV promoter in the context of Ad vectors in all the cell lines tested, including nonhuman primate and human cell lines. By using this murine CMV promoter, the investigators observed that the Ad vector dose required to achieve sufficient transduction could be reduced 100-fold and the cellular inflammation and viral cytotoxicity associated with the delivery of Ad vectors into the rat brain could be completely eliminated.

In the context of HD Ad vectors, the inclusion of a human centromeric region and a matrix attachment region as in-cis acting elements improved the maintenance of the Ad vector genome and the transgene expression level [57]. It can be concluded that even for the Ad vectors that have an extremely high transduction efficiency, the in-cis acting elements can be optimized to obtain maximized transgene expression.

4.6. Integrating Ad vectors

For replication-deficient Ad vectors, the existence of episomal Ad DNA presents the disadvantage of not integrating for long-term expression of the delivered foreign gene. In the case that stable genetic alternation needs to be maintained in dividing cells, integrating vectors are at present the tools of choice. Though integration is not a guarantee of stable transcription, since transgene expression from integrated vector genomes can be gradually silenced over time in some cases [161], at present the best way to get long-term transgene expression is to integrate the foreign gene into the host genome.

To incorporate integration machinery to Ad, a variety of hybrid vectors combining the highly efficient DNA delivery of Ad with the integrating machinery of adeno-associated viruses (AAV), retroviruses, and transposons have been emerging [162]. The unique biology of AAV has stimulated considerable efforts toward the development of AAV-based integrating Ad vectors. Such integrating Ad-AAV hybrid vectors devoid of all Ad viral genes were successfully developed by Lieber et al. [163] and Recchia et al. [164]. Lieber's hybrid Ad-AAV vectors displayed in vitro an integration frequency comparable to that reported for AAV vectors, and high transgene expression at a level comparable to the first-generation Ad vectors [163].

Baun's group did extensive studies exploiting the retrovirus integrating machinery to realize permanent Ad-delivered transgene expression. By flanking the transgene cassette in the Ad vector with the cis-acting elements from Moloney murine leukemia virus in a unique arrangement, they obtained high integration frequencies (between 5% and 11%) in rat spleen cells [165]. In consideration of the high transduction efficiency, this integration rate should be high enough to stably keep transgene expression at therapeutic levels for many gene therapy applications.

Kay and colleagues addressed the challenge of genome persistence by exploiting the promiscuous integration capabilities of the Sleeping Beauty (SB) transposon in Ad-mediated gene delivery [166]. SB transposon is a member of the Tc1/mariner superfamily of transposons, and undergoes cut-and-paste transposition through a DNA intermediate, mediated by the SB transposase [167]. By incorporating the SB integration machinery into an HD Ad vector containing human coagulation factor IX, therapeutic levels of human coagulation factor IX were sustained for more than 6 months in mice undergoing extensive liver proliferation [166].

However, at the current time there are great concerns about the risk of insertional mutagenesis due to random integration of vectors into the host genome. The recent clinical trial in France using

retroviral vectors in a gene therapy approach for X-linked severe combined immunodeficiency disease (X-SCID) had been taken as the milestone success in human gene therapy [168]. Unfortunately, two of the 11 patients treated during this X-SCID trial developed a leukemia-like disorder [169,170]. Now it is known that the cause of induced leukemia in X-SCID trial is attributed to the retrovirus vector integration in proximity to the LMO2 proto-oncogene promoter, which leads to aberrant transcription and expression of LMO2 [171]. Recent study suggests that the AAV vector preferentially integrates into active genes, though at present it is not clear if this will contribute to the risk of developing a malignancy [172]. Recchia's Ad/AAV hybrid vector system showed 35% of DNA integration was site-specific at the AAVS1 site in hepatoma cells, with one HD Ad vector expressing Rep78 and the second HD vector carrying a transgene flanked by AAV-ITRs [164]. However, excess Rep expression is toxic to the cells and may result in rearrangement of AAVS1 without transgene integration [173]. To avoid potential mutation risks of random integration, episomally replicating Ad vectors might be a potential alternative choice for long-term expression. Leblois et al. [174] inserted a loxP flanked Epstein-Barr virus (EBV) replicon into an Ad vector. Expression of Cre recombinase from a second Ad vector has been shown to release the transcription unit that could replicate coordinately during the cell cycle. Kreppel et al. [175] incorporated the episomally replicating machinery to an HD vector by utilizing the DNA recombinase FLPe to circularize the genome containing the EBV replicon, and obtained long-term transgene expression in proliferating cells. However, data of episomally replicating Ad vectors from in vivo studies are lacking.

4.7. Serotype switch or animal Ad vectors for readministration

In many gene therapy applications, lifelong expression might be needed, which would require readministration of vectors following the eventual loss of therapeutic transgene expression if the vectors have no integration or episomal replication machinery. However, readministration of an Ad vector will require the circumvention of the humoral immune response directed against the original vector capsid. Studies indicated that fiber-substituted Ad5 vectors containing fiber proteins of another serotype could not evade the humoral immune response against Ad5 [115], because hexon proteins of the capsid are the major targets of host-neutralizing antibodies in Ad5 infection [115,176,177]. This suggests that capsid partially modified Ad5 vectors do not meet the long-term need. Hence, the most practical approach to overcoming the obstacle of pre-existing antibodies is the use of alternative serotypes.

Morral et al. [178] observed in baboons that the hurdle of readministration due to the humoral response to an Ad5 vector was overcome by use of an Ad2 vector expressing hAAT. Their data further suggest that long-term expression of transgenes should be synergized by combining the reduced immunogenicity and toxicity of HD vectors with sequential delivery of vectors of different serotypes. However, it might be difficult to completely avoid cross-humoral response between Ad2 and Ad5 in all species including humans, as both Ads belong to the same subgroup and have high homogenicity. To address this issue, we and other groups have developed Ad35 vectors (subgroup B) [179–182] and have found that antibodies to Ad5 vector do not affect the transduction efficiency by Ad35 vector (Sakurai et al. unpublished observation, 181). Furthermore, while there is more than a 50% prevalence in adult humans of neutralizing antibodies to Ad5, less than 10% of individuals have anti-Ad35 neutralizing antibodies [181].

Since many humans are pre-exposed to human Ad, Ads from non-humans have been developed as vector systems for gene delivery. Mouse [183], avian [184], bovine [185], canine [186], porcine [187] and chimpanzee [188] Ad have been tested as vectors for various applications to overcome the barriers of pre-existing neutralizing antibodies. These animal-derived Ad vectors might be useful in evading humoral immune responses to human Ad5 for readministration. However, prior to their clinical application, safety issues such as oncogenicity should be addressed.

Readministration might be a solution to the transient transgene expression of Ad vectors when it is necessary. To balance the gain and loss in safety, efficacy and economy are the key points. The potential serotypes and animal origin Ads are limited.

Furthermore, this approach needs a complex set of clinical products.

5. Summary

Among all the vectors for gene delivery, no single one can meet all the requirements for all gene therapy applications. Similarly, for Ad vectors, no single approach can overcome the hurdle of immunogenic toxicities. It seems that HD vectors might serve as basis for combination with other approaches. It is clear that the ultimately ideal Ad vectors will have low or no immunogenic toxicities, and specifically transduce interested tissues/cells, with high, persistent and regulatable transgene expression. It is also clear that there is still a long way before research reaches this ideal.

Acknowledgements

Our work described in this review was supported by grants from the Ministry of Health, Labour and Welfare of Japan, and a Grant-in-aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. Z-L. Xu is a recipient of a post-doctoral fellowship from Japan Health Sciences Foundation.

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Mini Review

改良型アデノウイルスベクターを用いた造血幹細胞，間葉系幹細胞，ES細胞への高効率遺伝子導入

水口裕之[1)]，川端健二[1)]，櫻井文教[1)]，早川堯夫[2)]

[1)]独立行政法人医薬基盤研究所 遺伝子導入制御プロジェクト

[2)]独立行政法人医薬品医療機器総合機構

Efficient gene transfer into hematopoietic stem cell, mesenchymal stem cell, and ES cell by modified adenovirus vectors

Efficient gene transfer into stem cells which are able to self-renew and differenciate into certain type of cell is essential for not only difining the precise molecular mechanism of self-renewal and differenciation, but the supplying of the cells for regenerative medicine. In this paper, we review our approach to the efficient gene transfer into hematopoietic stem cell, mesenchymal stem cell, and ES cell by modified adenovirus vectors.

Rec.11/30/2004, Acc.2/4/2005, pp447-451

Hiroyuki Mizuguchi[1)], Kenji Kawabata[1)], Fuminori Sakurai[1)] and Takao Hayakawa[2)]

[1)]Laboratory of Gene Transfer and Regulation, National Institute of Biomedical Innovation

[2)]Pharmaceuticals and Medical Devices Agency

Key words adenovirus vector, gene transfer, hematopoietic stem cell, mesenchymal stem cell, ES cell

はじめに

造血幹細胞，間葉系幹細胞，ES細胞（多能性幹細胞）をはじめとする幹細胞は，自己複製能を有する一方で，多くの種類の細胞を産生する多分化能を有することから，再生医療のための細胞ソースとして注目されている．また，近年の研究によりこれら幹細胞の自己複製能の維持や，特定の細胞への分化能の獲得に関与する遺伝子が次々と同定され，細胞分化を自在に制御することも可能になりつつある．細胞増殖・分化の分子機構の解明には外来遺伝子を導入して発現させたり，あるいは特定の遺伝子の発現を抑制させたりすることが必要であり，効率の良い遺伝子導入法は必要不可欠である．これまでは，造血幹細胞や間葉系幹細胞，ES細胞への遺伝子導入にはレトロウイルスベクターやレンチウイルスベクターが汎用されてきたが，これらのベクターによる遺伝子発現は染色体への導入遺伝子の組み込みを伴うため長期的・永続的なものとなり，細胞分化が起こった後も導入遺伝子の発現は続くことになる．細胞分化に関わる遺伝子の中には，分化完了後は発現（あるいは抑制）を必要としない場合も多くあることが考えられ，このような場合には一過性の遺伝子発現（抑制）をもたらすベクター系が好ましいことになる．アデノウイルスベクターは，導入遺伝子が染色体外でエピソーマルに存在し自律複製することはないため，一過性の遺伝子発現を示し，接着系細胞をはじめとする多くの細胞種に対して効率良く遺伝子導入できることが知られている．しかしながら，造血幹細胞，間葉系幹細胞，ES細胞への遺伝子導入効率は低く，これらの細胞への適用には不向きであった．筆者らが開発を進め

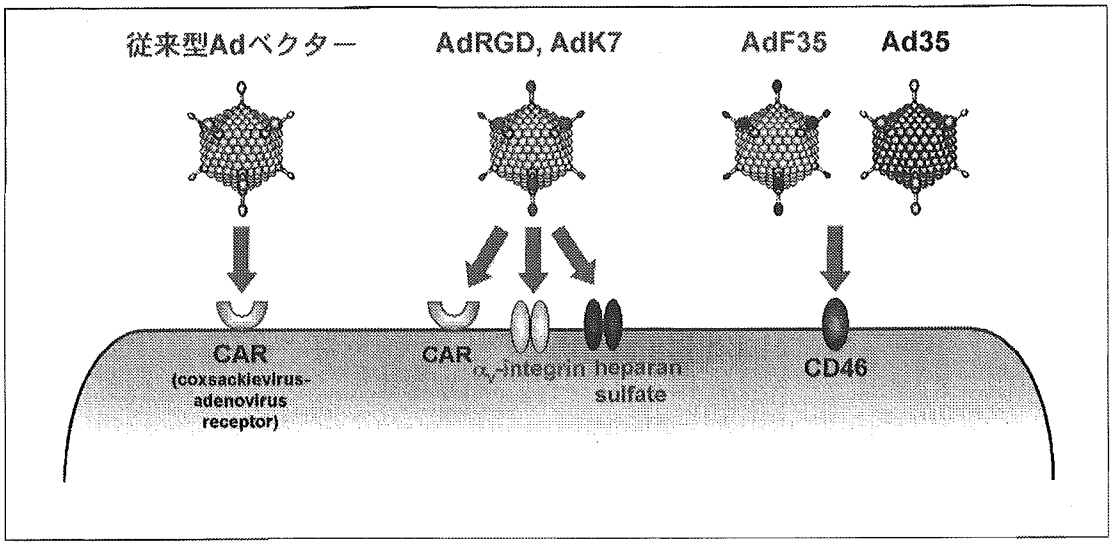

図1 改良型アデノウイルスベクターによる遺伝子導入

野生型のファイバーを持った従来型の5型アデノウイルスベクターは細胞表面上の受容体であるCARを認識して感染するが，RGD配列やポリリジン（K7：リジンが7つ続く）配列をファイバーに有したファイバー改変ベクター(AdRGD, AdK7)はCARだけでなくαvインテグリンやヘパラン硫酸を認識しても感染できる．また，ファイバー部分をサブグループBの35型アデノウイルスのファイバーに置換したベクター(AdF35)や，全ての構造タンパク質が35型アデノウイルスからなるベクター(Ad35)は，CD46を認識して感染する．

ている改良型アデノウイルスベクターは，これらの幹細胞へも効率良く遺伝子導入でき，分化機構解明などの基礎研究や，再生医療や遺伝子治療のための基盤技術になりうると期待される．

改良型アデノウイルスベクター

遺伝子治療や遺伝子導入用ベクターとして汎用されているアデノウイルスベクターは，サブグループCに属するヒト5型アデノウイルスを基盤としている（ヒトアデノウイルスはAからFまでのサブグループに分けられ，計51種の血清型が存在する）．5型アデノウイルスの感染には，ウイルスカプシドタンパク質のファイバーと，細胞表面上のCAR(coxsackievirus and adenovirus receptor)との結合を必要とするため，従来のアデノウイルスベクターはCAR陽性細胞へは効率良く遺伝子導入できるが，CARの発現が乏しい細胞への遺伝子導入効率は低いことが課題であった．CARの発現が乏しい細胞としては，造血幹細胞やT細胞をはじめとする血液系細胞，間葉系幹細胞，樹状細胞，一部の癌細胞（特に悪性度の高い癌細胞），血管内皮細胞，滑膜細胞などが知られており，このような細胞へはアデノウイルスベクターの適用は不向きであった．

筆者らは，ファイバータンパク質の外来ペプチド挿入部位として適したHIループやC末端コード領域に簡単に外来ペプチドコード遺伝子を挿入できるファイバー改変アデノウイルスベクター作製法を開発済みであり[1,2]，この技術と*in vitro*ライゲーションに基づいたE1欠損領域への外来遺伝子挿入法[3,4](Clontech社よりキット化）を合わせることにより，感染時のCAR依存性を克服した種々の改良型アデノウイルスベクターを簡単に作製することが可能となった．本法を用いて作製したファイバータンパク質のHIループやC末端領域にRGD (Arg-Gly-Asp)ペプチドやポリリジンペプチドを挿入したベクターでは，多くの細胞で発現しているαvインテグリンやヘパラン硫酸を認識して効率良く遺伝子導入することが可能となった[1,2](図1)．また，ファイバータンパク質を，CD46を受容体とする35型アデノウイルス（サブグループBに属する）由来のものに置換したベクター[5]や，全ての構造タンパク質が35型アデノウイルスからなるベクターも開発済みである[6,7](図1)．補体制御因子として知られているCD46は，ヒト由来の細胞ではほとんどの細胞で発現していることが知られており，35型アデノウイルスベクター（あるいはファイバー部分が35型アデノウイルスからなる5型アデノウイルスをベースにしたベクター）は，ヒト由来細胞への有力なベクターである．

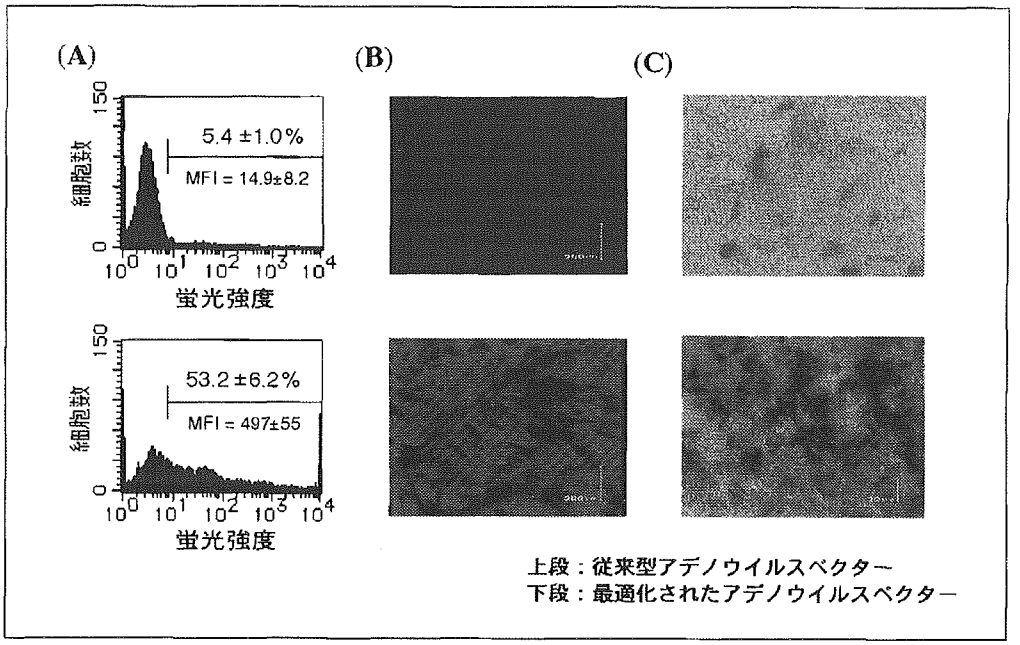

図2 改良型アデノウイルスベクターによる造血幹細胞(A)，間葉系幹細胞(B)，ES細胞(C)への高効率遺伝子導入

(A)EGFP(enhanced green fluorescence protein)を発現する5型（従来型）アデノウイルスベクター（上段）あるいは35型アデノウイルスベクター（下段）（ともにCMVプロモーター）を，ヒト骨髄由来CD34陽性細胞へ作用させ(MOI=300; plaque forming unit/cell=300)，EGFPの発現をフローサイトメーターで調べた．グラフ内の数字はEGFP陽性細胞の割合(%)，および平均蛍光強度(mean fluorescence intensity: MFI)を示す．(B)β-ガラクトシダーゼを発現する5型（従来型）アデノウイルスベクター（上段；3000 vector particle/cell）あるいはポリリジンペプチドを付与したファイバー改変アデノウイルスベクター（下段；1000 vector particle/cell）（ともにβアクチンプロモーター/CMVエンハンサー）を，初代培養ヒト間葉系幹細胞へ作用させ，β-ガラクトシダーゼの発現をX-gal染色で検討した．ポリリジンペプチドを付与したファイバー改変アデノウイルスベクターでは100%の細胞がX-gal陽性であったが，従来型アデノウイルスベクターでは改良型ベクターの3倍濃度を作用させてもX-gal陽性はわずかであった．(C)CMVプロモーター（上段）あるいはEF1αプロモーター（下段）でβ-ガラクトシダーゼを発現する従来型アデノウイルスベクターを，マウスES細胞（フィーダー細胞フリーの系）へ作用させ(1000 vector particle/cell)，β-ガラクトシダーゼの発現をX-gal染色で検討した．EF1αプロモーターを用いた場合，約90%以上の細胞がX-gal陽性であった．

改良型アデノウイルスベクターによる高効率遺伝子導入

1)造血幹細胞への遺伝子導入

ヒト造血幹細胞を含む画分であるCD34陽性細胞（本総説ではヒト造血幹細胞を含む画分であるCD34陽性細胞をあえて造血幹細胞として記述させていただく）では，CAR陽性細胞は数%と少なく，5型アデノウイルスベクターによる遺伝子導入効率も4～5%と極めて低い[6]．一方，ほぼ全てのCD34陽性細胞はCD46を発現しており，35型アデノウイルスベクターを用いると50%以上の細胞に目的遺伝子を発現させることができる[7](図2A)．理由は不明であるが（レセプターのCD46を発現しているにもかかわらず），CD34陽性細胞への35型アデノウイルスベクター（種々のプロモーターを搭載したもので検討）の作用濃度を上げても60%以上の遺伝子発現効率を得ることは困難であった．そこで，GFP発現を指標に，35型アデノウイルスベクターで作用後のCD34陽性細胞をGFP陽性細胞と陰性細胞に分取して，それぞれの細胞分画中のアデノウイルスゲノム量を定量的PCR法で測定したところ，GFP陰性細胞にも陽性細胞と同程度のウイルスゲノムが存在していた[8]．すなわち，約4割のCD34陽性細胞は，ウイルスは感染できるが遺伝子発現に至らないことが明らかとな

り，CD46の発現以外に遺伝子発現を決定する何らかの要因があることが示唆された．

2)間葉系幹細胞への遺伝子導入

間葉系幹細胞における分化機構解明などの基礎研究には，細胞株が利用されることが多いが，最終的には初代培養細胞で機能を検証する必要があることは言うまでもない．しかしながら，ヒト間葉系幹細胞もCARの発現が乏しく，従来の5型アデノウイルスベクターでの遺伝子導入効率は極めて低い．筆者らのヒト初代培養間葉系幹細胞を用いた検討では，従来型アデノウイルスベクターに比べ，RGDペプチドを付与したアデノウイルスベクターでは約16倍，35型ファイバーを付与したベクターでは約130倍，ポリリジンペプチドを付与したベクターでは約460倍の遺伝子発現効率を示し，100%の細胞で目的遺伝子を発現させることが可能であった（図2B）[9]．遺伝子発現期間に関しては継代培養を行わず培養を続けた場合は，1ヵ月以上にわたって遺伝子発現はほとんど低下せず，高レベルの発現を維持していた（継代培養を行い，細胞を常に分裂させて培養した場合は，細胞分裂に伴ってウイルスゲノムが希釈され，遺伝子発現は低下する）．

3)ES細胞への遺伝子導入

マウスES細胞への遺伝子導入には，プラスミドを用いたリポフェクションを用い，導入遺伝子が染色体に組み込まれたわずかの細胞を薬剤耐性遺伝子を用いて選択する方法が汎用されている．また，ポリオーマのlarge T抗原を発現させたES細胞の場合には，ポリオーマの複製起点をプラスミドに付与することでプラスミドが染色体外で複製し，遺伝子導入細胞を効率良く選択することが可能となる．これらはES細胞が細胞株であることを利用した外来遺伝子発現法であるが，前述のように，これらの遺伝子導入系では細胞分化の後も遺伝子発現が続くことによる問題点を伴う．

アデノウイルスベクターはこれまでES細胞への遺伝子導入には用いられてこなかったが，マウスES細胞におけるCARの発現を検討したところ陽性であり，アデノウイルスはマウスES細胞に感染できることが判明した[10]．ところが，多くの遺伝子発現実験で用いられ強力な発現を示すことが知られているCMV（サイトメガロウイルス）プロモーターでは，ほとんど全く遺伝子発現を誘導せず，アデノウイルスベクターがマウスES細胞には遺伝子導入できないと信じられていたことは，ウイルスの細胞へのエントリーに問題があるのではなく，CMVプロモーターが機能しないことに依っていることが明らかとなった．各種のプロモーターを用いてアデノウイルスベクターによるマウスES細胞での外来遺伝子発現を検討したところ，EF1 α (elongation factor 1α)プロモーターを用いれば最も効率良く遺伝子発現させることができ，単回のアデノウイルスベクターの作用で，約90%以上のマウスES細胞へ外来遺伝子を発現させることが可能であった(図2C)．また，ES細胞の未分化性維持に必須の転写因子であるSTAT3 (Signal Transducers and Activators of Transcription 3)[11]の機能を，STAT3のドミナントネガティブ体(STAT3F)をアデノウイルスベクターを用いて発現させることによって阻害させたところ，細胞は分化すること，この作用はNanog（ES細胞の分化多能性を維持できる必須転写因子）[12,13]を発現させることでレスキュー（STAT3Fを発現させても未分化性を維持）できることを実証した[10]．したがって，一過性に単回のベクター作用で細胞集団全体（遺伝子導入細胞を選択するのではなく）としての機能を検討したい場合には，アデノウイルスベクターは有力なツールになると期待される．なお，CMVプロモーターでRGDやポリリジンペプチドを付与したファイバー改変アデノウイルスベクターを用いれば，ES細胞へは遺伝子は導入されているが発現せず，フィーダー細胞（CAR陰性）では目的遺伝子を発現するため，フィーダー細胞への選択的な遺伝子発現を得ることができる．ES細胞は増殖が早く，細胞分裂に伴ってウイルスゲノムが希釈されるため，1000 vector particle/cellの条件下でアデノウイルスベクターを作用させた場合，LacZ陽性細胞は遺伝子導入12日後にはほぼ消失した[10]（8日後ではLacZ陽性細胞は認められた）．

4)幹細胞以外の細胞への遺伝子導入

従来の遺伝子導入法ではほとんど遺伝子導入できなかったが，改良型アデノウイルスベクターを用いることによって劇的な遺伝子導入効率の改善が認められる代表的な細胞として樹状細胞があげられる．RGDペプチドを付与したファイバー改変アデノウイルスベクターでは，ヒト末梢血単球由来樹状細胞やマウス骨髄由来樹状細胞に対し，90～95%以上の高効率での遺伝子導入が可能であり[14,15]，樹状細胞を用いた基礎研究や免疫細胞治療（樹状細胞ワクチン）に向けた応用研究にとって必要不可欠なツールとなりつつある．

また，従来の5型アデノウイルスベクターでは，遺伝子導入が困難であった様々な細胞株についても，多くの場合ファイバー改変ベクターを用いることで遺伝子導入効率の改善が期待できる．例えば脂肪細胞分化研究に汎用されているマウス3T3-L1細胞では，ポリリジンペプチドを付与したファイバー改変ベクターを用いれば，90%以上の細胞に遺伝子を導入，発現させることができる[16]（従来型アデノウイルスベクターでは数%の遺伝子導入効率しか示さない）．

おわりに

各種幹細胞への一過性の遺伝子導入は，改良型アデノウイルスベクターを用いることによって劇的に効率が改善され，目的にもよるが，遺伝子導入細胞のソーティングや選択を行わなくても，単回のベクター作用で細胞分化の制御などに利用可能な段階になった．今後，筆者らの開発したこれらのベクターが，幹細胞研究や再生医療研究などの基礎·応用研究に大きく貢献できることを期待している．

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カプシドタンパク質を改変した改良型アデノウイルスベクターによる高効率遺伝子導入

Efficient Gene Transfer by Capsid-Modified Improved Adenovirus Vectors

水口裕之[*1]　早川堯夫[*2]

アデノウイルスベクターは既存の遺伝子治療用ベクターの中では最も遺伝子導入効率に優れているが，アデノウイルス受容体（CAR）を発現している細胞にしか遺伝子導入できないこと，ベクターの感染域に組織特異性がないことが課題となっている。筆者らは，これらの問題点を克服し，CAR非依存的に高効率に遺伝子導入できるベクターや，ターゲティング能を有した改良型アデノウイルスベクターの開発を進めている。

1. はじめに

アデノウイルスベクターは遺伝子導入効率に優れていることから，がんをはじめとする疾患に対する遺伝子治療臨床研究や，遺伝子機能解析などを目的とした基礎研究に汎用されている。しかしながら，従来のアデノウイルスベクター（2型あるいは5型アデノウイルスを基盤としている）による遺伝子導入には，標的細胞にアデノウイルス受容体（CAR：coxsackievirus-adenovirus receptor）の発現を必要とするため，CARの発現が乏しい細胞（造血幹細胞をはじめとする血液系細胞，間葉系幹細胞，樹状細胞，血管内皮細胞，血管平滑筋細胞，骨格筋細胞，滑膜細胞，多くのマウス由来の細胞株など）へはアデノウイルスベクターが適用できないことが問題となっている。また，がん細胞は悪性度の進行と共に，CARの発現レベルが低下することが報告されており[1, 2]，アデノウイルスベクターを用いてがんを対象とした遺伝子治療臨床研究を進める上で考慮すべき問題となっている。

筆者らのグループでは，このような問題を克服できるベクターとして，CARを利用しなくても効率良く遺伝子導入できる改良型アデノウイルスベクターの開発を進めている。本稿では，感染域を改変したアデノウイルスベクターの開発とその応用例を紹介すると共に，カプシドタンパク質を改変することで，組織特異性を有したターゲティングアデノウイルスベクターの開発状況について簡単に紹介する。なお，アデノウイルスベクターに関する基本的な解説は，筆者らの過去の総説[3, 4]などを参考にしていただきたい。

2. カプシドタンパク質を改変した改良型アデノウイルスベクターの開発

アデノウイルスベクターによる遺伝子導入時のCAR依存性を克服するために，ファイバータンパク質を改変した改良型ベクターの開発が進んで

[*1]Hiroyuki Mizuguchi　㈱医薬基盤研究所
[*2]Takao Hayakawa　国立医薬品食品衛生研究所　副所長

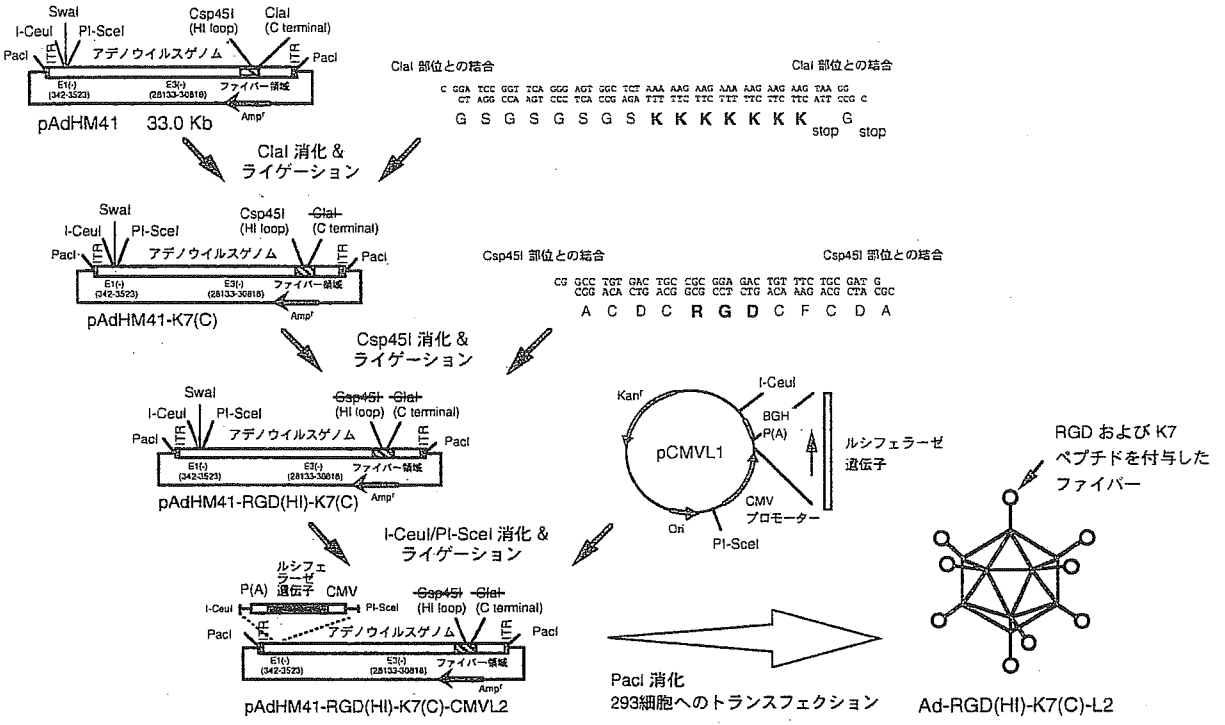
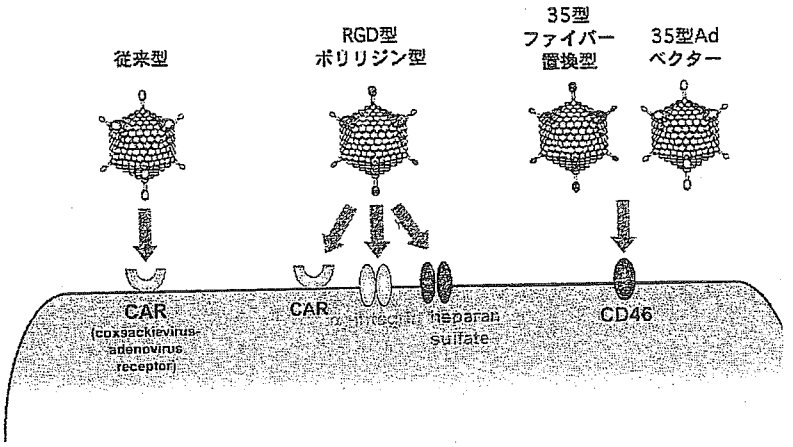


図1　ファイバー改変アデノウイルスベクターの作製法

ファイバーノブの HI ループあるいは C 末端をコードする遺伝子配列部分にユニークな制限酵素である Csp45I あるいは ClaI 部位（それぞれ）をもったベクタープラスミド pAdHM41 を両酵素で切断し，挿入したいペプチド（この場合 RGD 配列およびポリリジン配列）に相当する合成オリゴ DNA を *in vitro* ライゲーションで導入する。その後，I-CeuI と PI-SceI 部位を利用して *in vitro* ライゲーションでルシフェラーゼ遺伝子を E1 欠損部位に挿入する。生じたプラスミドをウイルスゲノム両末端に存在する PacI 部位で切断し，293 細胞にトランスフェクションすると，RGD 配列とポリリジン配列をファイバーに有するルシフェラーゼ発現アデノウイルスベクターができる。

いる。ファイバーはテール，シャフト，ノブの 3 領域からなり，ノブ領域が CAR と結合する。筆者らは，ファイバーノブの外来ペプチドの挿入部位として適した HI ループや C 末端コード領域に，1 ステップの *in vitro* ライゲーションで任意の外来ペプチドコード遺伝子を挿入できるシステムを開発しており，極めて簡便に様々なペプチドをファイバーに表現した改良型アデノウイルスベクターが作製できるようになった[5, 6]（図 1）。本ファイバー改変アデノウイルスベクター作製法を用いて，αv インテグリンに親和性がある RGD（Arg-Gly-Asp）ペプチドや，ヘパラン硫酸に親和性があるポリリジンペプチドをファイバー表面上に遺伝子工学的に表現させることにより，CAR を発現していない細胞に対しても効率良く遺伝子導入できるベクターの作製も可能となった[5, 6]（図 2）。これらのベクターは，多くの細胞で発現しているαv インテグリンやヘパラン硫酸を認識して感染できることから，様々な細胞種や広範な目的への適用が期待できる。

ファイバー改変アデノウイルスベクターとしては，ファイバー領域だけを CAR 以外の分子を受容体としている 5 型アデノウイルスとは異なった血清型のアデノウイルス（3・11・35 型など sub-group B に属するアデノウイルス）由来のファイバーに置換したベクターも開発されている。筆者らは，ファイバー部分を 35 型アデノウイルス由来のファイバーに置き換えたアデノウイルスベクター，および全ての構造タンパク質を 35 型アデノウイルス由来したベクターを開発しているが[7~9]，これらのベクターは赤血球を除くほぼ全てのヒト由来細胞で発現が認められる CD46 を認識して感染できるため，ヒト由来細胞をターゲットとする場合には極めて有効である。しかし，醤



野生型のファイバーを持った従来型の5型アデノウイルスベクターは細胞表面上の受容体であるCARを認識して感染するが，RGD配列やポリリジン配列をファイバーに有したファイバー改変ベクターはCARだけでなくαvインテグリンやヘパラン硫酸を認識しても感染できる。また，35型のアデノウイルスのファイバーを有したベクターや，全ての構造タンパク質が35型アデノウイルスからなるベクターは，CD46を認識して感染する。

図2　ファイバー改変アデノウイルスベクター

歯類由来の細胞はCD46を発現しておらず，ほとんど遺伝子導入できないこと，本ベクターの*in vivo*での機能を評価する小動物（マウス，ラットなど）のモデルがなく（齧歯類由来の細胞へは遺伝子導入できないため），遺伝子治療への応用を目的とした場合には課題点となっている。後者の問題を克服するために，筆者らはヒトCD46発現トランスジェニックマウスをモデル系として用いることで，35型アデノウイルスベクター（あるいは35型アデノウイルス由来のファイバーを有したベクター）の機能を検討中である。

感染域を変更する他のアプローチとして，ファイバーノブ部分をファイバータンパク質と同様に3量体を形成するレオウイルスの表面タンパク質であるσ1に置き換え，σ1が認識するjunctional adhesion molecule 1（JAM1）を認識して遺伝子導入できるようなアデノウイルスベクターも開発されている[10]。

3. 改良型アデノウイルスベクターによる高効率遺伝子導入（応用例）

カプシドタンパク質を改変した改良型アデノウイルスベクターによる高効率遺伝子導入の応用例として，遺伝子治療や再生医療（細胞治療）で重要な細胞への適用について以下に簡単に紹介する。改良型アデノウイルスベクターを用いることで，従来は遺伝子導入が困難であった多くの細胞種に対して効率の良い遺伝子導入が可能となっており，基礎研究や治療を目的とした応用研究に極めて重要な基盤技術になっている。

3.1　間葉系幹細胞

間葉系幹細胞は再生医療のための細胞ソースとして注目されており，細胞分化関連遺伝子を導入して目的細胞を分化誘導したり，治療用タンパク質を産生させるように細胞機能を改変した間葉系幹細胞を治療へ応用する場合には，高効率の遺伝子導入系が不可欠である。しかしながら，ヒト間葉系幹細胞はCARの発現が乏しく，従来の5型アデノウイルスベクターでの遺伝子導入効率は極めて低い。筆者らのヒト初代培養間葉系幹細胞を用いた検討では，従来型アデノウイルスベクターに比べ，RGDペプチドを付与したアデノウイルスベクターでは10数倍，35型ファイバーを付与したベクターでは200〜300倍，ポリリジンペプチドを付与したベクターでは約1,000倍の遺伝子発現効率を示した。ヒト間葉系幹細胞への遺伝子導入にはポリリジン型の改変アデノウイルスベクターが最適であり，100%の細胞で目的遺伝子を発現させることが可能である（**写真1**）。

3.2　CD34陽性細胞

ヒト造血幹細胞を含む画分であるCD34陽性細胞では，CAR陽性細胞は数%と少なく，5型アデノウイルスベクターによる遺伝子導入効率も4〜5%と極めて低い。一方，ほぼ全てのCD34陽性細胞はCD46を発現しており，35型アデノウイルスベクターを用いると50%以上の細胞に目

的遺伝子を発現させることができる[8]。RGD型やポリリジン型の改変アデノウイルスベクターは，CD34陽性細胞への遺伝子導入には適していない。

3.3 樹状細胞

樹状細胞は生体防御を担う最も強力な抗原提示細胞であり，遺伝子改変を加えて樹状細胞機能を高めた細胞療法は，がんなどへの難治性疾患に対する新しい治療法として期待されている。しかしながら，従来の遺伝子導入法では樹状細胞への遺伝子導入も困難であることが知られている。アデノウイルスベクターを用いた場合も，ヒトおよびマウス樹状細胞（ヒト末梢血単球由来樹状細胞やマウス骨髄由来樹状細胞）への遺伝子導入効率は10%以下と低いが，RGD型の改変アデノウイルスベクターでは，ほぼ100%の遺伝子導入効率を示す[11, 12]。ヒト樹状細胞へは，35型ファイバーを有したベクターも有効である。

3.4 その他

脂肪細胞分化研究に汎用されている細胞株である3T3-L1マウス脂肪前駆細胞や脂肪細胞への遺伝子導入には，ポリリジン型の改変アデノウイルスベクターが極めて有効である[13]（**写真2**）。ヒト間葉系幹細胞から分化誘導した脂肪細胞に対しては35型ファイバーを有したベクターが有効である。

また，メラノーマに対しては一般にRGD型のベクターが適している[6, 14]。

4. ターゲティングアデノウイルスベクターの開発

標的細胞指向性を有したベクターの開発は，遺伝子治療の有効性と安全性の向上のために重要な

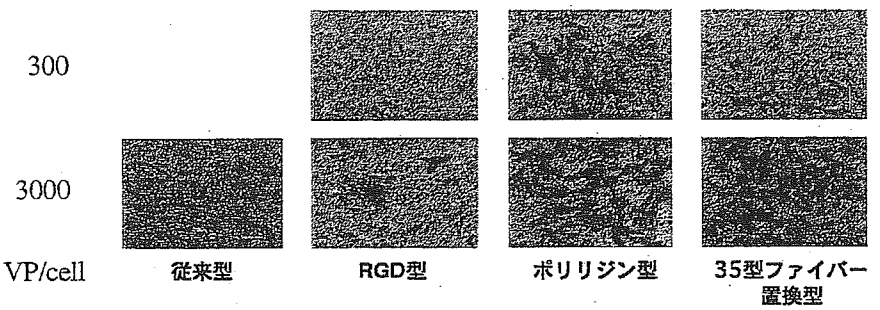


写真1 間葉系幹細胞に対する各種ファイバー改変アデノウイルスベクターの遺伝子発現効率

ヒト初代培養間葉系幹細胞に対して，βガラクトシターゼ（LacZ）を発現する各種ファイバー改変アデノウイルスベクター（従来型，RGD型，ポリリジン型，35型ファイバー置換型）を300または1,000 VP（vector particle）/cellの条件下で作用させ，2日後X-gal染色を行った。

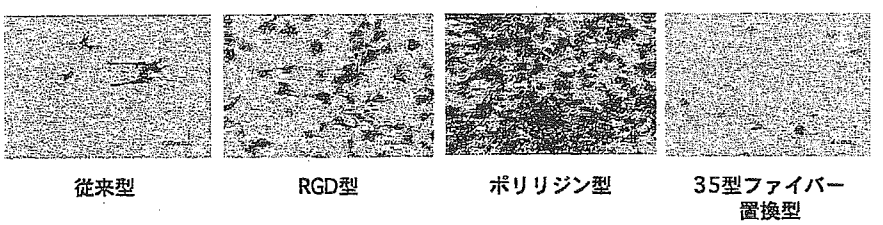


写真2 3T3-L1脂肪前駆細胞に対する各種ファイバー改変アデノウイルスベクターの遺伝子発現効率

マウス3T3-L1細胞に対して，LacZを発現する各種ファイバー改変アデノウイルスベクター（従来型，RGD型，ポリリジン型，35型ファイバー置換型）を10,000 VP/cellの条件下で作用させ，2日後X-gal染色を行った。