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Obstacles to Human Hematopoietic Stem Cell Transduction by Recombinant Adeno-Associated Virus 2 Vectors

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Abstract Recombinant adeno-associated virus 2 (AAV) vectors have proven to be a potentially useful alternative to the more commonly used retroviral and adenoviral vectors for gene therapy in humans. Their safety and efficacy in Phase I clinical trials for gene therapy of cystic fibrosis and hemophilia B have been well documented, and their remarkable versatility and efficacy in a wide variety of pre-clinical models of human diseases have catapulted these vectors to the forefront. AAV vectors have been shown to be particularly well suited for transduction of brain and muscle cells. However, controversies exist with regard to their utility as a vector for gene transfer into human hematopoietic stem cells. On the one hand, some investigators have concluded that AAV vectors do not transduce hematopoietic stem cells at all, and others have reported that stem cell transduction requires enormously high vector-to-cell ratios. On the other hand, some investigators have reported high-efficiency transduction of human hematopoietic stem cells at low vector-to cell ratios. This article will provide a historical perspective as well as attempt to elaborate the reasons behind these controversies which have become clearer by studies focused on understanding, at the molecular level, the fundamental aspects of the life cycle of recombinant AAV vectors. J. Cell. Biochem. Suppl. 38: 39–45, 2002.

Key words: AAV; vectors; stem cells; gene transfer; gene therapy

The adeno-associated virus 2 (AAV)-based vectors have gained attention as an alternative to the more commonly used retrovirus- and adenovirus-based vectors primarily because of the non-pathogenic nature of the wild-type (wt) AAV [Muzyczka, 1992; Berns and Giraud, 1996]. Recombinant AAV vectors have been shown to transduce certain cell types, such as brain and muscle, exceedingly well [Kaplitt et al., 1994; Xiao et al., 1996]. However, controversies exist with regard to the efficacy of AAV vectors in transducing hematopoietic stem cells. There are three different viewpoints. First, AAV vectors do not transduce primary $CD34^+$ progenitor cells [Alexander et al., 1997]. Second, $CD34^+$ cells can be transduced by AAV vectors, but extremely high multiplicities-of-infection (MOI) are required [Malik et al., 1996; Hargrove et al., 1997; Nathwani et al., 2000]. And third, AAV vectors can efficiently transduce $CD34^+$ cells at relatively low MOIs [Ponnazhagan et al., 1997; Chatterjee et al., 1999]. Recent studies have unraveled most, if not all, of the confounding factors responsible for the genesis of these controversies, and a clearer picture has now emerged. A brief historical perspective follows.

Zhou et al. [1994] first reported successful transduction of human $CD34^+$ cells by recombinant AAV vectors. These studies were subsequently corroborated by a number of investigators [Goodman et al., 1994; Miller et al., 1994; Walsh et al., 1994; Fisher-Adams et al.,

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1996; Luhovy et al., 1996]. However, Alexander et al. [1997] failed to observe efficient infection of these cells, and attributed the low-level transduction to pseudotransduction mediated by contaminants in the vector stocks.

In order to address this discrepancy, Ponnazhagan et al. [1997] undertook a systematic study in which $CD34^+$ cells from twelve different hematologically normal volunteer donors were either mock-infected, or infected with 100 MOI of a recombinant AAV-*lacZ* vector under identical conditions and analyzed for *lacZ* gene expression by fluorescence-activated cell sorting (FACS). These results are shown in Table I. Of the twelve donors studied, $CD34^+$ cells from six showed no detectable activity of *lacZ* gene expression, whereas the transduced gene expression could be readily detected in cells from the remaining six donors. However, among the six positive donor samples, the level of the transduced *lacZ* gene expression varied significantly and ranged between 15 and 80%.

In ^{35}S-labeled AAV-binding experiments, it was also documented that, whereas the virus could bind to $CD34^+$ cells from a positive donor, little binding occurred with $CD34^+$ cells from a negative donor under identical conditions. Similarly, viral DNA entry assays revealed that viral entry occurred in $CD34^+$ cells that were positive for *lacZ* gene expression, but not in $CD34^+$ cells that did not show expression of the *lacZ* gene. These results prompted Ponnazhagan et al. [1996] to further reinforce their contention that AAV infection of human cells involved a putative cellular receptor [Ponnazhagan et al., 1994]. This proposal was based on the fact that the first human cell type had been identified that could not be infected by the wt AAV, or transduced by a number of different recombinant AAV vectors [Ponnazhagan et al., 1996].

TABLE I. Donor Variation in the Level of the Transduced *lacZ* Gene Expression in $CD34^+$ Cells

Donor	% Cells expressing the *lacZ* gene
1	1
2	80
3	50
4	37
5	0
6	0
7	15
8	0
9	0
10	2
11	53
12	29

Equivalent number (1.5×10^5) of $CD34^+$ cells from each donor (50% females, 50% males; average age 25 years) were transduced with the same stock of a recombinant AAV-*lacZ* vector at an MOI of 100 transducing units ($\sim 1 \times 10^7$ particles) per cell, and 48 h post-infection, percentage of cells expressing the *lacZ* gene, compared with mock-transduced controls, was determined by FACS [Ponnazhagan et al., 1997].

The search for the putative cell surface receptor for AAV infection intensified, and in 1998, cell surface heparan sulfate proteoglycan (HSPG) was identified as a cellular receptor for AAV [Summerford and Samulski, 1998]. The ubiquitous expression of HSPG, perhaps, accounts for the broad host-range of AAV. Bartlett et al. [1999] subsequently documented that AAV infectivity correlated strongly with HSPG expression. Thus, the conclusion that $CD34^+$ cells are non-permissive for AAV infection [Alexander et al., 1997] is erroneous since analyzing $CD34^+$ cells from a limited number of donors can be misleading given the significant donor variation in terms of receptor expression [Ponnazhagan et al., 1997].

More recent studies by Qing et al. [1999] and Summerford et al. [1999] led to the identification of human fibroblast growth factor (FGF) receptor 1 (FGFR1) and $\alpha V\beta 5$ integrin, respectively, as putative cell surface co-receptors for efficient infection by AAV. These studies led to the conclusion that following initial attachment of AAV to the cell surface via HSPG receptor, efficient entry of the virus requires the presence of a putative cellular co-receptor. Based on all the available information, a model for AAV infection has been proposed, which is depicted in Figure 1. In this model, co-expression of cell surface HSPG and FGFR1 is required for successful AAV binding followed by viral entry. Thus, the lack of expression of HSPG and/or FGFR1 might in some cases account for the lack of transduction of hematopoietic stem cells by AAV vectors. Qing et al. [1999] also documented that AAV binding and infection are strongly inhibited by FGF, which might also contribute to the inefficient transduction if AAV infection is carried out in the presence of serum, which contains FGF.

Qing et al. [1999] also documented that AAV binds to murine NIH3T3 cells efficiently, but little transgene expression occurs. This observation was originally interpreted as the inability of AAV to enter these cells. However, Hansen et al. [2000] documented that the lack of transgene expression in NIH3T3 cells was not due to

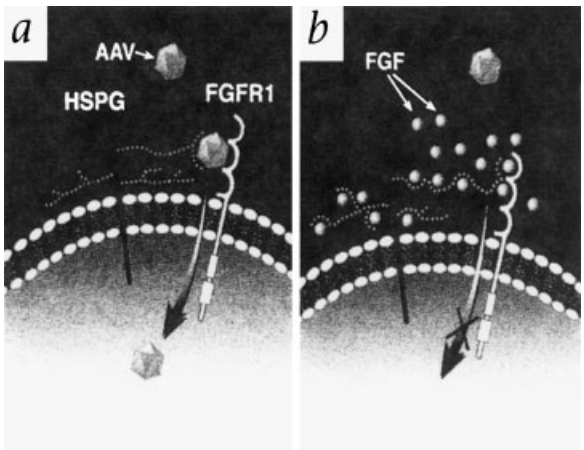

Fig. 1. A model for the role of cell surface HSPG and FGFR1 in mediating AAV binding and entry into the host cell. Co-expression of HSPG and FGFR1 and/or αVβ5 integrin (not shown) is required for successful binding of AAV followed by viral entry into a susceptible cell (**Panel a**), both of which are perturbed by the ligand, bFGF, which also requires the HSPG-FGFR1 interaction (**Panel b**). [Qing et al., 1999]

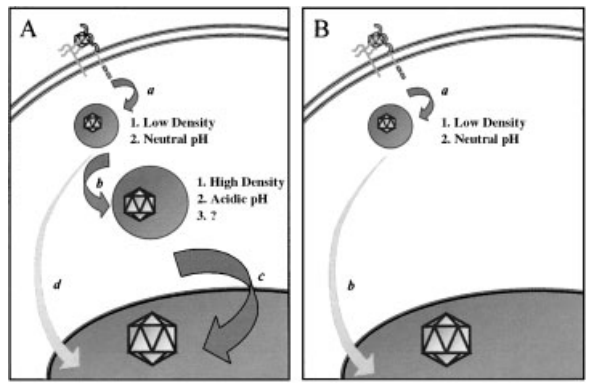

Fig. 2. A model for intracellular trafficking of AAV in permissive (**Panel A**) and semi-permissive (**Panel B**) cells. AAV binds and enters the early endosomes of both cell types efficiently (**a**). In permissive cells, most of the virions progress down the endocytic pathway (**b**), enter a dense endocytic organelle with a low pH, undergo a putative capsid modification (**?**), and subsequently enter the nucleus by an unknown mechanism (**c**). This process can be blocked by inhibitors of endosomal acidification in which case virions enter the nucleus by a less efficient pathway (**d**). In contrast, virions in semi-permissive cells fail to pass through dense, acidic endosomes, and therefore, do not traffick efficiently to the nucleus. Instead, AAV escapes from early endosomes and inefficiently enters the nucleus by an alternate route (**b**). [Hansen et al., 2001]

the lack of viral entry as determined by both Southern blot analysis of viral genomes and by using fluorescently-labeled AAV. They further documented that despite successful entry, a significant fraction of AAV vectors failed to enter the nucleus in NIH3T3 cells as determined by Southern blot analysis of the viral genomes in the cytoplasmic and nuclear fractions. Hansen et al. [2000] also concluded that AAV transduction efficiency correlates well with the extent of viral trafficking into the nucleus. Hansen et al. [2001] subsequently documented that in permissive cells, AAV escapes the early endosomes prior to efficient transport across the nuclear membrane, whereas in less permissive cells, AAV fails to do so. These observations are summarized in a model shown in Figure 2. When these studies were extended to include primary murine $Sca1^{+}, lin^{-}$ hematopoietic cells from C57Bl6 donor mice, which were either mock-infected or infected with a recombinant AAV vector containing the CMV promoter-driven enhanced green fluorescence protein (EGFP) reporter gene and transplanted into lethally-irradiated congenic recipient mice, a significant fraction of AAV genomes failed to gain entry into the nucleus in spleen cell colonies (CFU-S) harvested twelve days post-transplantation [Tan et al., 1999]. Thus, impaired intracellular trafficking to the nucleus might account, in part, for the observed lack of high-efficiency transduction of hematopoietic stem cells by AAV vectors.

In 1996, studies from two independent laboratories suggested that AAV second-strand DNA synthesis is a rate-limiting step in efficient transduction by AAV vectors [Ferrari et al., 1996; Fisher et al., 1996]. Qing et al. [1997] reported the identification of a cellular protein that interacts with the single-stranded D-sequence in the AAV inverted terminal repeat (ITR). This protein, designated the single-stranded D-sequence-binding protein (ssD-BP), was shown to be phosphorylated at tyrosine residues by epidermal growth factor receptor protein tyrosine kinase (EGFR PTK) [Mah et al., 1998], and the tyrosine phosphorylated state of the ssD-BP was shown to correlate well with the transduction efficiency of AAV vectors in human cells in vitro and murine tissues in vivo [Qing et al., 1998]. Qing et al. [2001] subsequently purified this protein and identified it to be a cellular protein that binds the immuno-suppressant drug FK506, termed the FK506-binding protein (FKBP52). FKBP52 was purified using a prokaryotic expression plasmid containing the human cDNA. FKBP52 has previously been shown to be phosphorylated

at serine/threonine residues. The purified protein could be phosphorylated at both tyrosine and serine/threonine residues, and only the phosphorylated forms of FKBP52 were shown to interact with the AAV single-stranded D-sequence probe. Furthermore, in in vitro DNA replication assays, the tyrosine-phosphorylated FKBP52 inhibited the AAV second-strand DNA synthesis by greater than 90%. The serine/threonine-phosphorylated FKBP52 caused ~40% inhibition, whereas the dephosphorylated FKBP52 had no effect on the AAV second-strand DNA synthesis. Deliberate overexpression of FKBP52 effectively reduced the extent of tyrosine-phosphorylation of this protein resulting in a significant increase in AAV-mediated transgene expression in human and murine cell lines. These studies corroborate that the phosphorylation status of the cellular FKBP52 protein correlates strongly with AAV transduction efficiency [Qing et al., 1998].

Based on all available data, Qing et al. [2001] have proposed a model, which is depicted in Figure 3. In this model, cellular FKBP52, phosphorylated at both tyrosine and/or serine/threonine residues, specifically interacts with the single-stranded D-sequence within the AAV-ITRs and blocks the viral second-strand DNA synthesis. The model predicts that tyrosine-phosphorylated FKBP52 is more inhibitory than that phosphorylated at serine/threonine residues. Infection with adenovirus, expression of adenovirus E4orf6 protein, or treatments with inhibitors of tyrosine and serine/threonine kinase inhibitors lead to dephosphorylation of FKBP52 which binds poorly to the D-sequence, thereby allowing viral second-strand DNA synthesis, and consequently, efficient transgene expression. These studies imply that the phosphorylation status of the cellular FKBP52 might account for the observed lack of high-efficiency transduction of hematopoietic stem cells by AAV vectors. Thus, in addition to the use of recombinant AAV vectors that might not be completely free of contaminating, wt AAV-like particles [Wang et al., 1998], efficient transduction of primary hematopoietic stem cells by AAV vectors might be limited by one or more of the following:

1. Lack of appropriate receptor/co-receptor expression for AAV binding and entry,
2. Lack of efficient intracellular trafficking of AAV to the nucleus, and

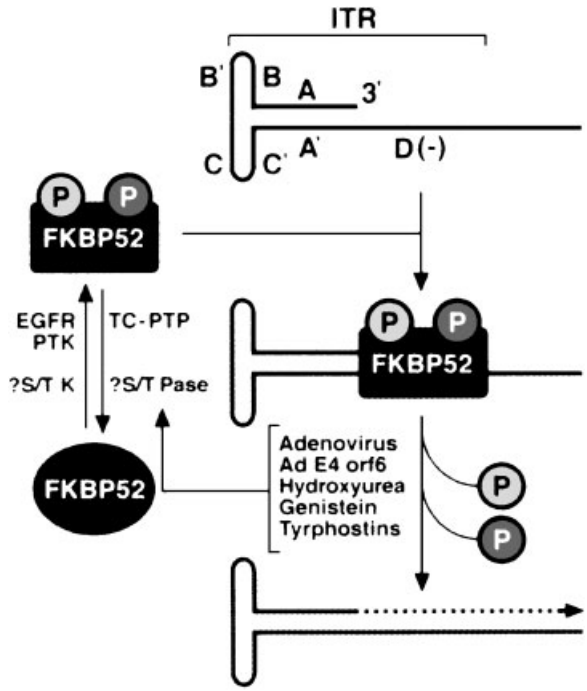

Fig. 3. A model for the role of the cellular FKBP52 protein in AAV second-strand DNA synthesis. FKBP52, phosphorylated either at tyrosine residues by the EGFR PTK, or at serine/threonine residues by an unknown cellular serine/threonine protein tyrosine kinase, interacts with the D(−) sequence in the AAV-ITR, and inhibits the viral second-strand DNA synthesis. Co-infection with adenovirus, expression of adenovirus E4orf6 protein, or treatments with inhibitors of tyrosine and serine/threonine kinase inhibitors lead to dephosphorylation of FKBP52 which can no longer bind to the D(−) sequence, thereby allowing the viral second-strand DNA synthesis, and consequently, efficient transgene expression. [Qing et al., 2001]

3. Lack of optimal conversion to transcriptionally-active double-stranded AAV genome.

The efficiency of conversion to the double-stranded form also has implications in the integration of the proviral genome with the host cell chromosomal DNA. Although some reports have implied that AAV-mediated transgene expression in $CD34^+$ cells is transient presumably because of less efficient integration [Malik et al., 1997; Nathwani et al., 2000], integration of the viral genome in human $CD34^+$ cells and their progenitors in vitro has been documented by polymerase chain reaction (PCR) [Goodman et al., 1994], Southern blotting [Fisher-Adams et al., 1996], as well as by fluorescence in situ hybridization (FISH) [Chatterjee et al., 1999] analyses. PCR-based assays have also been used to imply that long-term repopulating hematopoietic stem cells can be successfully

transduced by recombinant AAV vectors in murine and rhesus monkey models, respectively [Ponnazhagan et al., 1997; Schimmenti et al., 1998]. More recently, using Southern blot analyses of total genomic DNA from bone marrow cells obtained from recipient mice 14 months post-transplantation, Tan et al. [2001] have provided conclusive evidence that recombinant AAV vectors are capable of mediating high-efficiency, stable transduction of murine hematopoietic stem cells in vivo.

Thus, it has become increasingly clear that systematic studies have led to a better understanding of most, if not all, of the controversies with reference to hematopoietic stem cell transduction by recombinant AAV vectors. It is also clear that further studies focused on understanding, at the molecular level, the fundamental aspects of the life cycle of AAV vectors will be parlayed into optimal transduction of hematopoietic stem cells by recombinant AAV vectors. Some of these strategies include deliberate expression of HSPG and/or FGFR1 genes to allow high-efficiency of vector binding and entry, manipulation of specific cellular organelle structures to improve intracellular vector trafficking to the nucleus resulting in increased transduction, and modulation of the phosphorylation status of the cellular FKBP52 protein leading to highly efficient viral second-strand DNA synthesis and transgene expression as well as rapid conversion to duplex DNA, leading to high-efficiency integration of the proviral genome into the host chromosome resulting in stable transduction and long-term transgene expression.

Additional strategies, which have already been used, include the development of CD34-targeted vectors [Yang et al., 1998], the use of serotypes other than AAV2 [Handa et al., 2000], and pseudotytping the recombinant AAV genomes into the human parvovirus B19 capsids [Ponnazhagan et al., 1998; Weigel-Kelley et al., 2001].

In all, in contrast to the currently prevalent view that recombinant AAV vectors are unlikely to be useful for hematopoietic stem cell transduction [Russell and Kay, 1999; van Os et al., 1999; Halene and Kohn, 2000; Van Tendeloo et al., 2001], a more complete understanding of the virus–host cell interactions will ultimately lead to the development of additional strategies to achieve high-efficiency transduction of these cells by AAV vectors, which in turn, will prove to be a safer alternative to the more commonly used retroviral vectors in hematopoietic stem cell gene therapy applications.

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REFERENCES

Alexander IA, Russell DW, Miller AD. 1997. Transfer of contaminants in adeno-associated virus vector stocks can mimic transduction and lead to artifactual results. Hum Gene Ther 8:1911–1920.

Bartlett JS, Kleinschmidt J, Boucher RC, Samulski RJ. 1999. Targeted adeno-associated vector transduction of nonpermissive cells mediated by a bispecific F(ab'γ)$_2$ antibody. Nat Biotechnol 17:181–186.

Berns KI, Giraud A. 1996. Biology of adeno-associated virus. Curr Top Microbiol Immunol 218:1–23.

Chatterjee S, Li W, Wong CA, Fisher-Adams G, Lu D, Guha M, Macer JA, Forman SJ, Wong KK Jr, 1999. Transduction of primitive human marrow and cord blood-derived hematopoietic progenitor cells with adeno-associated virus vectors. Blood 93:1882–1894.

Ferrari FK, Samulski T, Shenk T, Samulski RJ. 1996. Second-strand synthesis is a rate-limiting step for efficient transduction by recombinant adeno-associated virus vectors. J Virol 70:3227–3234.

Fisher KJ, Gao G-P, Weitzman MD, DeMatteo R, Burda JF, Wilson JM. 1996. Transduction with recombinant adeno-associated virus for gene therapy is limited by leading-strand synthesis. J Virol 70:520–532. 1996.

Fisher-Adams G, Wong KK Jr., Podsakoff GM, Forman SJ, Chatterjee S. 1996. Integration of adeno-associated virus vectors in CD34$^+$ human hematopoietic progenitor cells after transduction. Blood 88:492–504.

Goodman S, Xiao X, Donahue RE, Moulton A, Miller JL, Walsh CE, Young NS, Samulski RJ, Nienhuis AW. 1994. Recombinant adeno-associated virus mediated gene transfer into hematopoietic progenitor cells. Blood 84:1492–1500.

Halene S, Kohn DB. 2000. Gene therapy using hematopoietic stem cells: Sisyphus approaches the crest. Hum Gene Ther 11:1259–1267.

Handa A, Muramatsu S-I, Qiu J, Mizukami H, Brown KE. 2000. Adeno-associated virus (AAV)-3-based vectors transduce haematopoietic cells not susceptible to transduction with AAV-2-based-vectors. J Gen Virol 81:2077–2084.

Hansen J, Qing KY, Kwon H-J, Mah C, Srivastava A. 2000. Impaired intracellular trafficking of adeno-associated

virus type 2 vectors limits efficient transduction of murine fibroblasts. J Virol 74:992–997.

Hansen J, Qing KY, Srivastava A. 2001. Adeno-associated virus type 2-mediated gene transfer: Altered endocytic processing enhances transduction efficiency in murine fibroblasts. J Virol 75:4080–4090.

Hargrove PW, Vanin EF, Kurtzman GJ, Nienhuis AW. 1997. High-level globin gene expression mediated by a recombinant adeno-associated virus genome that contains the 3' γ globin gene regulatory element and integrates as tandem copies in erythroid cells. Blood 89: 2167–2175.

Kaplitt MG, Leone P, Samulski RJ, Xiao X, Pfaff DW, O'Malley KL, During MJ. 1994. Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. Nat Genet 8:148–153.

Luhovy M, McCune S, Dong J-Y, Prchal JF, Townes TM, Prchal JT. 1996. Stable transduction of recombinant adeno-associated virus into hematopoietic stem cells from normal and sickle cell patients. Biol Blood Marrow Transpl 2:24–30.

Mah C, Qing KY, Khuntrirat B, Ponnazhagan S, Wang X-S, Kube DM, Yoder MC, Srivastava A. 1998. Adeno-associated virus 2-mediated gene transfer: Role of epidermal growth factor receptor protein tyrosine kinase in transgene expression. J Virol 72:9835–9841.

Malik P, McQuiston SA, Yu X-J, Pepper KA, Krall WJ, Podsakoff GM, Kurtzman GJ, Kohn DB. 1997. Recombinant adeno-associated virus mediates a high level of gene transfer but less efficient integration in K562 human hematopoietic cell line. J Virol 71:1776–1783.

Miller JL, Donahue RE, Sellers SE, Samulski RJ, Young NS, Nienhuis AW. 1994. Recombinant adeno-associated virus (rAAV)-mediated expression of human γ-globin gene in human progenitor-derived erythroid cells. Proc Natl Acad Sci USA 91:10183–10187.

Muzyczka N. 1992. Use of adeno-associated virus as a general transduction vector for mammalian cells. Curr Top Microbiol Immunol 158:97–129.

Nathwani AC, Hanawa H, Vandergriff J, Kelly P, Vanin EF, Nienhuis AW. 2000. Efficient gene transfer into human cord blood $CD34^+$ cells and the $CD34^+$ $CD38^-$ subset using highly purifed recombinant adeno-associated viral vector preparations that are free of helper virus and wild-type AAV. Gene Ther 7:183–195.

Ponnazhagan S, Wang X-S, Kang LY, Woody MJ, Nallari ML, Munshi NC, Zhou SZ, Srivastava A. 1994. Transduction of human hematopoietic cells by the adeno-associated virus 2 vectors is receptor-mediated. Blood 84:742a.

Ponnazhagan S, Wang X-S, Woody MJ, Luo F, Kang LY, Nallari ML, Munshi NC, Zhou SZ, Srivastava A. 1996. Differential expression in human cells from the p6 promoter of human parvovirus B19 following plasmid transfection and recombinant adeno-associated virus 2 (AAV) infection: Human megakaryocytic leukaemia cells are non-permissive for AAV infection. J Gen Virol 77: 1111–1122.

Ponnazhagan S, Yoder MC, Srivastava A. 1997. Adeno-associated virus type 2-mediated transduction of murine hematopoietic cells with long-term repopulating ability and sustained expression of a human globin gene in vivo. J Virol 71:3098–3104.

Ponnazhagan S, Weigel KA, Raikwar SP, Mukherjee P, Yoder MC, Srivastava A. 1998. Recombinant human parvovirus B19 vectors: Erythroid cell-specific delivery and expression of transduced genes. J Virol 72:5224–5230.

Qing KY, Wang X-S, Kube DM, Ponnazhagan S, Bajpai A, Srivastava A. 1997. Role of tyrosine phosphorylation of a cellular protein in adeno-associated virus 2-mediated transgene expression. Proc Natl Acad Sci USA 94:10879–10884.

Qing KY, Khuntrirat B, Mah C, Kube DM, Wang X-S, Ponnazhagan S, Zhou SZ, Dwarki VJ, Yoder MC, Srivastava A. 1998. Adeno-associated virus type 2-mediated gene transfer: Correlation of tyrosine phosphorylation of the cellular single-stranded D sequence-binding protein with transgene expression in human cells in vitro and murine tissues in vivo. J Virol 72:1593–1599.

Qing KY, Mah C, Hansen J, Zhou SZ, Dwarki VJ, Srivastava A. 1999. Human fibroblast growth factor receptor 1 is a co-receptor for infection by adeno-associated virus 2. Nat Med 5:71–77.

Qing KY, Hansen J, Weigel-Kelley KA, Tan MQ, Zhou SZ, Srivastava A. 2001. Adeno-associated virus type 2-mediated gene transfer: Role of cellular FKBP52 protein in transgene expression. J Virol 75:8968–8976.

Russell DW, Kay MA. 1999. Adeno-associated virus vectors and hematology. Blood 94:864–874.

Schimmenti S, Boesen J, Claassen EAW, Valerio D, Einerhand MPW. 1998. Long-term genetic modification of rhesus monkey hematopoietic cells following transplantation of adeno-associated virus vector-transduced $CD34^+$ cells. Hum Gene Ther 9:2727–2734.

Summerford C, Samulski RJ. 1998. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus 2 virions. J Virol 72:1438–1445.

Summerford C, Bartlett J, Samulski RJ. 1999. αVβ5 integrin: A co-receptor for adeno-associated virus 2 infection. Nat Med 5:78–81.

Tan MQ, Qing KY, Yoder MC, Srivastava A. 1999. Adeno-associated virus 2-mediated transduction of primary murine hematopoietic progenitor cells in vitro and in vivo. Blood 94:356a.

Tan MQ, Qing KY, Zhou SZ, Yoder MC, Srivastava A. 2001. Adeno-associated virus 2-mediated transduction and erythroid lineage-restricted, long-term expression of the normal human β-globin gene in hematopoietic cells from homozygous β-thalassemic mice. Mol Ther 3:940–946.

Van Os R, Avraham H, Banu N, Mauch PM, Whater J, Yang Y, Du B. 1999. Recombinant adeno-associated virus-based vectors provide short-term rather than long-term transduction of primitive hematopoietic stem cells. Stem Cells 17:117–120.

Van Tendeloo VFI, Van Broeckhoven C, Berneman ZN. 2001. Gene therapy: Principles and applications to hematopoietic cells. Leukemia 15:523–544.

Walsh CE, Nienhuis AW, Samulski RJ, Brown MG, Miller JL, Young NS, Liu JM. 1994. Phenotypic correction of Fanconi anemia in human hematopoietic cells with a recombinant adeno-associated virus vector. J Clin Invest 94:1440–1448.

Wang X-S, Khuntirat B, Qing KY, Ponnazhagan S, Kube DM, Zhou SZ, Dwarki VJ, Srivastava A. 1998. Characterization of wild-type adeno-associated virus type

2-like particles generated during recombinant viral vector production and strategies for their elimination. J Virol 72:5472–5480.

Weigel-Kelley KA, Yoder MC, Srivastava A. 2001. Recombinant human parvovirus B19 vectors: Erythrocyte P antigen is necessary but not sufficient for successful transduction of human hematopoietic cells. J Virol 75: 4110–4116.

Xiao X, Li J, Samulski RJ. 1996. Efficient long-term gene transfer into muscle tissue of immunocompetent mice by adeno-associated virus vector. J Virol 70:8098–8108.

Yang Q, Mamounas M, Yu G, Kennedy S, Leaker B, Merson J, Wong-Staal F, Yu M, Barber JR. 1998. Development of novel cell surface CD34-targeted recombinant adeno-associated virus vectors for gene therapy. Hum Gene Ther 9:1929–1937.

Zhou SZ, Cooper S, Kang LY, Ruggieri L, Heimfeld S, Srivastava A, Broxmeyer HE. 1994. Adeno-associated virus 2-mediated high efficiency gene transfer into immature and mature subsets of hematopoietic progenitor cells in human umbilical cord blood. J Exp Med 179: 1867–1875.