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ADDENDUM

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Vascular-targeted recombinant adeno-associated viral vectors for the treatment of rare diseases

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ABSTRACT

There is a lack of treatment options for many rare genetic disorders. Gene therapy represents a promising and innovative approach to fill this gap. One of such rare disorders is incontinentia pigmenti caused by X-linked deletions or mutations in the *Nemo* gene. The disease affects the skin, teeth, and eyes and, most importantly, it leads to a severe vascular pathology of the central nervous system. The genetic treatment of vascular disorders such as incontinentia pigmenti critically depends on safe and efficient gene delivery. Thus, focus has been set on the development of suitable vector systems. In a recent issue of *EMBO Molecular Medicine,* we describe the development of a recombinant adeno-associated viral (AAV) vector with a unique tropism for the brain vascular endothelium (termed AAV-BR1) and, as a proof of principle that may be transferred to other vascular disorders, report on its therapeutic application in a mouse model of incontinentia pigmenti. Here, we discuss the implications of our findings and further highlight the promising prospects as well as potential limitations of such vectors.

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AAV vectors for systemic application

Among the various viruses that are used as vector systems, AAV has emerged as one of the most favorable candidates for gene therapy.[1] The introduction of Glybera® (alipogene tiparvovec), being approved as first gene therapy drug of the Western world by European regulatory authorities in 2012, emphasizes the importance of AAV as a gene therapy vector. In addition, Glybera® as medication for lipoprotein lipase deficiency (an orphan disease with an incidence of below 1: 1,000,000) indicates that gene therapy of rare diseases can be interesting both from a medical and a pharmacoeconomic point of view.[2,3] Although the various AAV serotypes differ in their tropism, none of the natural occurring AAV serotypes is specific for one single tissue or cell type.[4] Thus, most AAV vectors must be administered locally to the target tissue to enhance efficacy and minimize side effects. Systemic vector administration, however, is favorable for many diseases, especially those affecting large organs (such as lung and brain), or those having a disseminated pathology (such as metastatic cancer). For gene therapy of primary vascular or vascular-mediated diseases such as hypertension, stroke, vascular dementia or multiple sclerosis, systemic vector administration might even be a mandatory prerequisite. Yet, systemic vector administration critically relies on safe, efficient and target-specific vectors. The ongoing discussion about hepatocellular carcinoma potentially being induced by AAV highlights the importance of target-specific gene delivery.[5,6] In our recent study published in *EMBO Molecular Medicine*, we report on the generation of a novel recombinant AAV vector, termed AAV-BR1, which specifically targets the brain and the spinal cord where it predominantly transduces blood-brain barrier (BBB)-associated endothelial cells after intravenous injection in mice.[7] Several systems have been developed and employed to generate AAV vectors with improved tropism, but only very few vectors have been proven to really be suited for systemic target-specific gene therapy.

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The vascular endothelium as therapeutic target

Apart from unspecific (and functionally silent) particle uptake in the reticular endothelial system of the spleen, AAVBR1 has been shown to specifically home to the brain after intravenous injection in mice. Even more importantly, the strong AAV-BR1-mediated transgene expression was restricted to the brain, and to lesser extent the spinal cord and the eyes. At a cellular level, AAV-BR1 predominantly transduced the BBB-associated endothelium and sporadically some scattered neurons. The endothelial transduction could be reconfirmed *in vitro* on primary murine cells and, albeit weaker, on immortalized human cerebral microvascular endothelial cells (hCMEC/D3), a well characterized cell line with blood-brain barrier (BBB) properties.[8] Interestingly, the transgene expression *in vivo* was stable for the entire live span of mice (animals were sacrified before their natural death at day 665 after vector injection to collect their organs). The ability to mediate such long lasting transgene expression in the brain vascular endothelium renders AAV-BR1 very promising for gene therapy of neurovascular disorders with the aim to improve cerebral perfusion. As key players, endothelial cells are also the main target to modulate the BBB or to study the barrier's functions in an experimental context.[9] Although not examined in our *EMBO Molecular Medicine* study, AAV-BR1 might potentially also be used to treat non-vascular neurological disorders. In a previous study, it was shown that AAV-mediated delivery of therapeutic transgenes to brain endothelial cells can ameliorate the pathology in mouse models of non-vascular-related, rare neurological diseases (mucopolysaccharidosis type VII and neuronal ceroid lipofuscinosis).[10] Due to the close proximity of endothelial cells and neurons, the secretion of the transgene products (β-glucuronidase and tripeptidyl peptidase I) from vector-transduced endothelial cells into the brain parenchyma presumably was sufficient to induce the observed therapeutic effects. Thus, in our view, there is reason to believe that vectors such as AAV-BR1 will be well suited for similar approaches.

Treatment of incontinentia pigmenti

To test the therapeutic applicability of our AAV-BR1 vector, we chose a mouse model of incontinentia pigmenti, a rare X-linked genetic disorder that mainly affects the skin (also the nails, the hair, the teeth and the eyes) and, most importantly, is accompanied by severe neurological impairments.[11] Deletions or mutations in the NF-κB essential modulator (NEMO), a key component of the NF-κB signal cascade, can lead to death of brain endothelial cells, resulting in empty tubes of basement membranes, so-called string vessels, cerebral hypoperfusion, a disrupted BBB, and epileptic seizures.[12] Since there is no conventional therapy for this disorder, we chose to employ the AAV-BR1 vector to substitute the missing genetic information in neonatal heterozygous mice with germline deletion of the X-chromosomal *Nemo* gene ($Nemo^{-/}+$) and in adult animals with a conditional knockout of the *Nemo* gene in brain endothelial cells ($Nemo^{beKO}$). Using the AAV-BR1-NEMO vector, we were able to significantly ameliorate the cerebrovascular pathology of incontinentia pigmenti and to protect the brain endothelial cells, seen as normalization of total vessel length, reduction of string vessels to a normal level, and reduced extravasation of albumin and immunoglobulin into the brain parenchyma. Importantly, we could show that transduction of brain endothelial cells by the viral vector AAV-BR1 has no negative effects on the integrity of the BBB, underlying the safety of this approach. With regard to these promising results, we are currently investigating the effect of our gene therapy vector on other clinically relevant parameters such as epileptiform events and seizures in the incontinentia pigmenti mouse model.

Technical issues: Generating targeted vectors from random AAV-display peptide libraries

Most approaches to redirect the tropism of AAV rely on the modification of the viral capsid. One common straight forward approach is the genetic incorporation of or the chemical coupling to specific ligands (e.g., previously phage-selected peptides) or antibodies, as reviewed in refs. 13, 14. This approach, however, has the disadvantage to rely on the preexisting availability of specific targeting agents, i.e. ligands or antibodies which can lose their specificity when being transferred into the AAV capsid. A promising alternative is an approach called "directed evolution," utilizing large pools of AAV capsid variants (AAV libraries) that are being screened *in vitro* or *in vivo* for desired properties. Such AAV libraries either consist of shuffled capsid proteins of different AAV serotypes, often accompanied by further random mutations, e.g. by error-prone PCR,

or they comprise pools of random peptides that are displayed on the AAV capsid in certain relevant positions.[15-19] In theory, such libraries allow to select for particles with the ability to target any unique structure on the cell surface.[15,20-22] The screening of random AAV display peptide libraries is the only approach to select peptides directly within the structural constraints of the assembled AAV capsid. Our brain-targeted vector AAVBR1 was isolated from such a random library with heptapeptide insertion in the capsid of AAV2 at a position known to be relevant for receptor binding (R588).[17] In our view there are few important variables which mainly determine the successful screening of a random AAV library:

i) the library design and the exact insertion site, as disruption of the natural receptor binding site is essential
ii) the diversity of the library, as a high diversity correlates with a high number of capsid variants to choose from
iii) the number of selection rounds, as in each round unspecific particles should decrease in favor of specific ones
iv) the technique to identify the relevant particles from the pool of remaining particles in the last selection round, as even after several successful selection rounds, the most common clone is not always the most specific one

The AAV2 display peptide library used to screen for brain vasculature-homing capsid variants had a diversity of approx. $2x10^8$ clones and AAV-BR1 was identified after 5 selection rounds with 2 d circulation time each. As the "NRGTEWD" peptide displayed by AAV-BR1 was part of the enriched peptide sequence motif (XX**G**XX**W**X), it was quickly identified as one of the most promising variants. However, although carefully considering all points mentioned above, in our experience the identification of the best suited capsids still can be difficult, as there might be no clear sequence motif. In addition, unspecific capsid variants with an advantage during library production might become strongly enriched during selection. Thus, we further improved the technique and recently described a screening system based on next-generation sequencing, accompanied by different rating scores to identify the most relevant particles after selecting random AAV display peptide libraries.[22]

Concluding remarks

There is huge potential for rare genetic disorders to benefit from gene therapy in the near future. Despite all drawbacks, our understanding of rare diseases is growing and the available gene therapy tools are getting better. Vector systems are being improved continuously, and AAV provides a very promising platform. In our recent study published in *EMBO Molecular Medicine*, we could show that the screening of random AAV display peptide libraries is a promising technique to generate truly tailored vectors.[7] The production of recombinant AAV2 vectors displaying the brain-targeted NRGTEWD peptide (AAV-BR1) yields comparable virus amounts to unmodified wildtype AAV2 in repetitive experiments (in our hands in approx. $1x10^{11}$ genomic particles from $1x10^7$ HEK 293T cells after iodixanol purification). Thus, the insertion of the NRGTEWD peptide does not seem to negatively influence capsid assembly, which is noteworthy since capsid-modified recombinant AAV vectors sometimes suffer from poor virus production. Although other AAV serotypes like AAV9 are known to yield >10 fold higher titers than AAV2, the amounts of recombinant AAV-BR1 that are needed for gene therapy approaches can easily be produced in few large scale preparations.[23] The tropism of our selected vector AAV-BR1 for BBB-associated endothelial cells is unique and well suited for the treatment of neurovascular diseases, which was proven by using AAV-BR1 to normalize many of the severe neurological impairments in a mouse model of incontinentia pigmenti. Still, there are several open questions that are worth to be investigated further. Up to now, we do not know which receptor/s is/are utilized by AAV-BR1 to specifically transduce brain endothelial cells. It has been shown that the transmembrane protein KIAA0319L is essential for cellular uptake of a variety of AAV serotypes, apart from glycans that are commonly used by AAV for primary attachment [24,25] Therefore, this protein might also be important for AAV-BR1. However, the sporadically observed AAV-BR1-transduced neurons indicate that AAV-BR1 crosses the BBB to some extent, presumably via transcytosis. Thus, it needs to be investigated which variables determine whether the vector is taken up by endocytosis or crosses the BBB via transcytosis. Experiments involving immunoprecipitation, microarrays or targeted knockdown/ overexpression of

candidate genes might help to answer these questions. Another important aspect might become relevant when thinking about the translation of the preclinical mouse studies into the clinic. Although we have shown that AAV-BR1 is able to transduce immortalized human brain endothelial cells (hCMEC/D3) *in vitro*, it is unclear whether the vector would also be effective in human patients *in vivo*, as the situation *in vitro* is not comparable to the *in vivo* situation. Taken into culture, cells can dedifferentiate and change their expression profile.[26,27] Potential inter-species differences might also play a role as Chen et al. reported of capsid-modified AAV vectors that even distinguish between mice with a lysosomal storage disorder and healthy litter mice of the same strain.[10] Nonetheless, the observed long-lasting and specific transgene expression mediated by AAV-BR1 is unique and the successful treatment of incontinenti pigmenti is very promising, encouraging further studies. The finding that target-specific particles can indeed be obtained by selecting random AAV display peptide libraries *in vivo* becomes even more important when considering that such a selection can be performed on a variety of organs or tissues, which we have recently confirmed in another study choosing the lung as target organ.[22]

Disclosure of potential conflicts of interest

The University Medical Center Hamburg-Eppendorf (UKE) has filed a patent application for the capsid-modified AAV vector BR1 on behalf of the authors. The authors have no additional competing financial interests.

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References

[1] Samulski RJ, Muzyczka N. AAV-Mediated Gene Therapy for Research and Therapeutic Purposes. Annu Rev Virol 2014; 1:427-51; PMID:26958729; http://dx.doi.org/10.1146/annurev-virology-031413-085355

[2] Morrison C. $1-million price tag set for Glybera gene therapy. Nat Biotechnol 2015; 33:217-8; PMID:25748892; http://dx.doi.org/10.1038/nbt0315-217

[3] Hegele RA, Ginsberg HN, Chapman MJ, Nordestgaard BG, Kuivenhoven JA, Averna M, Boren J, Bruckert E, Catapano AL, Descamps OS, et al. The polygenic nature of hypertriglyceridaemia: implications for definition, diagnosis, and management. Lancet Diabetes Endocrinol 2014; 2:655-66; PMID:24731657; http://dx.doi.org/10.1016/S2213-8587(13)70191-8

[4] Zincarelli C, Soltys S, Rengo G, Rabinowitz JE. Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection. Mol Ther 2008; 16:1073-80; PMID:18414476; http://dx.doi.org/10.1038/mt.2008.76

[5] Bell P, Moscioni AD, McCarter RJ, Wu D, Gao G, Hoang A, Sanmiguel JC, Sun X, Wivel NA, Raper SE, et al. Analysis of tumors arising in male B6C3F1 mice with and without AAV vector delivery to liver. Mol Ther 2006; 14:34-44; PMID:16682254; http://dx.doi.org/10.1016/j.ymthe.2006.03.008

[6] Nault JC, Datta S, Imbeaud S, Franconi A, Mallet M, Couchy G, Letouze E, Pilati C, Verret B, Blanc JF, et al. Recurrent AAV2-related insertional mutagenesis in human hepatocellular carcinomas. Nat Genet 2015; 47:1187-93; PMID:26301494; http://dx.doi.org/10.1038/ng.3389

[7] Körbelin J, Dogbevia G, Michelfelder S, Ridder DA, Hunger A, Wenzel J, Seismann H, Lampe M, Bannach J, Pasparakis M, et al. A brain microvasculature endothelial cell-specific viral vector with the potential to treat neurovascular and neurological diseases. EMBO Mol Med 2016; 8(6):609-25; PMID:27137490; http://dx.doi.org/10.15252/emmm.201506078

[8] Weksler BB, Subileau EA, Perriere N, Charneau P, Holloway K, Leveque M, Tricoire-Leignel H, Nicotra A, Bourdoulous S, Turowski P, et al. Blood-brain barrier-specific properties of a human adult brain endothelial cell line. FASEB J 2005; 19:1872-4; PMID:16141364

[9] Assmann JC, Korbelin J, Schwaninger M. Genetic manipulation of brain endothelial cells in vivo. Biochim Et Biophy Acta 2016; 1862:381-94; http://dx.doi.org/10.1016/j.bbadis.2015.10.006

[10] Chen YH, Chang M, Davidson BL. Molecular signatures of disease brain endothelia provide new sites for CNS-directed enzyme therapy. Nat Med 2009; 15:1215-8; PMID:19749771; http://dx.doi.org/10.1038/nm.2025

[11] Meuwissen ME, Mancini GM. Neurological findings in incontinentia pigmenti; a review. Eur J Med Genet 2012; 55:323-31; PMID:22564885; http://dx.doi.org/10.1016/j.ejmg.2012.04.007

[12] Ridder DA, Wenzel J, Muller K, Tollner K, Tong XK, Assmann JC, Stroobants S, Weber T, Niturad C, Fischer L, et al. Brain endothelial TAK1 and NEMO safeguard the neurovascular unit. J Exp Med 2015; 212:1529-49; PMID:26347470; http://dx.doi.org/10.1084/jem.20150165

[13] Michelfelder S, Trepel M. Adeno-associated viral vectors and their redirection to cell-type specific receptors. Adv Genet 2009; 67:29-60; PMID:19914449; http://dx.doi.org/10.1016/S0065-2660(09)67002-4

[14] Buchholz CJ, Friedel T, Buning H. Surface-engineered viral vectors for selective and cell type-specific gene delivery. Trend Biotechnol 2015; 33:777-90; http://dx.doi.org/10.1016/j.tibtech.2015.09.008

[15] Grimm D, Lee JS, Wang L, Desai T, Akache B, Storm TA, Kay MA. In vitro and in vivo gene therapy vector evolution via multispecies interbreeding and re-targeting of adeno-associated viruses. J Virol 2008; 82:5887-911; PMID:18400866; http://dx.doi.org/10.1128/JVI.00254-08

[16] Lisowski L, Dane AP, Chu K, Zhang Y, Cunningham SC, Wilson EM, Nygaard S, Grompe M, Alexander IE, Kay MA. Selection and evaluation of clinically relevant AAV variants in a xenograft liver model. Nature 2014; 506:382-6; PMID:24390344; http://dx.doi.org/10.1038/nature12875

[17] Müller OJ, Kaul F, Weitzman MD, Pasqualini R, Arap W, Kleinschmidt JA, Trepel M. Random peptide libraries displayed on adeno-associated virus to select for targeted gene therapy vectors. Nat Biotechnol 2003; 21:1040-6; PMID:12897791; http://dx.doi.org/10.1038/nbt856

[18] Perabo L, Buning H, Kofler DM, Ried MU, Girod A, Wendtner CM, Enssle R, Hallek M. In vitro selection of viral vectors with modified tropism: The adeno-associated virus display. Mol Ther 2003; 8:151-7; PMID:12842438; http://dx.doi.org/10.1016/S1525-0016(03)00123-0

[19] Varadi K, Michelfelder S, Korff T, Hecker M, Trepel M, Katus HA, Kleinschmidt JA, Muller OJ. Novel random peptide libraries displayed on AAV serotype 9 for selection of endothelial cell-directed gene transfer vectors. Gene Ther 2012; 19:800-9; PMID:21956692; http://dx.doi.org/10.1038/gt.2011.143

[20] Michelfelder S, Kohlschutter J, Skorupa A, Pfennings S, Muller O, Kleinschmidt JA, Trepel M. Successful expansion but not complete restriction of tropism of adeno-associated virus by in vivo biopanning of random virus display Peptide libraries. PLoS One 2009; 4:e5122; PMID:19357785; http://dx.doi.org/10.1371/journal.pone.0005122

[21] Ying Y, Muller OJ, Goehringer C, Leuchs B, Trepel M, Katus HA, Kleinschmidt JA. Heart-targeted adeno-associated viral vectors selected by in vivo biopanning of a random viral display peptide library. Gene Ther 2010; 17:980-90; PMID:20393510; http://dx.doi.org/10.1038/gt.2010.44

[22] Körbelin J, Sieber T, Michelfelder S, Lunding L, Spies E, Hunger A, Alawi M, Rapti K, Indenbirken D, Muller OJ, et al. Pulmonary Targeting of Adeno-associated Viral Vectors by Next-generation Sequencing-guided Screening of Random Capsid Displayed Peptide Libraries. Mol Ther 2016; 24(6):1050-61; PMID:27018516; http://dx.doi.org/10.1038/mt.2016.62

[23] Holehonnur R, Luong JA, Chaturvedi D, Ho A, Lella SK, Hosek MP, Ploski JE. Adeno-associated viral serotypes produce differing titers and differentially transduce neurons within the rat basal and lateral amygdala. BMC Neurosci 2014; 15:28; PMID:24533621; http://dx.doi.org/10.1186/1471-2202-15-28

[24] Mietzsch M, Broecker F, Reinhardt A, Seeberger PH, Heilbronn R. Differential adeno-associated virus serotype-specific interaction patterns with synthetic heparins and other glycans. J Virol 2014; 88:2991-3003; PMID:24371066; http://dx.doi.org/10.1128/JVI.03371-13

[25] Pillay S, Meyer NL, Puschnik AS, Davulcu O, Diep J, Ishikawa Y, Jae LT, Wosen JE, Nagamine CM, Chapman MS, et al. An essential receptor for adeno-associated virus infection. Nature 2016; 530:108-12; PMID:26814968; http://dx.doi.org/10.1038/nature16465

[26] Liaw L, Schwartz SM. Comparison of gene expression in bovine aortic endothelium in vivo versus in vitro. Differences in growth regulatory molecules. Arterioscler Thromb 1993; 13:985-93; PMID:7686396; http://dx.doi.org/10.1161/01.ATV.13.7.985

[27] Staton CA, Reed MW, Brown NJ. A critical analysis of current in vitro and in vivo angiogenesis assays. Inter J Exp Pathol 2009; 90:195-221; http://dx.doi.org/10.1111/j.1365-2613.2008.00633.x