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Nephron segment-specific gene expression using AAV vectors

Laureano D. Asico [a], Santiago Cuevas [a], Xiaobo Ma [a], Pedro A. Jose [a, b], Ines Armando [a], Prasad R. Konkalmatt [a, *]

[a] Department of Medicine, The George Washington University, Washington, DC, USA
[b] Department of Pharmacology and Physiology, The George Washington University, Washington, DC, USA

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ABSTRACT

AAV9 vector provides efficient gene transfer in all segments of the renal nephron, with minimum expression in non-renal cells, when administered retrogradely via the ureter. It is important to restrict the transgene expression to the desired cell type within the kidney, so that the physiological endpoints represent the function of the transgene expressed in that specific cell type within kidney. We hypothesized that segment-specific gene expression within the kidney can be accomplished using the highly efficient AAV9 vectors carrying the promoters of genes that are expressed exclusively in the desired segment of the nephron in combination with administration by retrograde infusion into the kidney via the ureter. We constructed AAV vectors carrying eGFP under the control of: kidney-specific cadherin (KSPC) gene promoter for expression in the entire nephron; Na^+/glucose co-transporter (SGLT2) gene promoter for expression in the S1 and S2 segments of the proximal tubule; sodium, potassium, 2 chloride co-transporter (NKCC2) gene promoter for expression in the thick ascending limb of Henle's loop (TALH); E-cadherin (ECAD) gene promoter for expression in the collecting duct (CD); and cytomegalovirus (CMV) early promoter that provides expression in most of the mammalian cells, as control. We tested the specificity of the promoter constructs *in vitro* for cell type-specific expression in mouse kidney cells in primary culture, followed by retrograde infusion of the AAV vectors via the ureter in the mouse. Our data show that AAV9 vector, in combination with the segment-specific promoters administered by retrograde infusion via the ureter, provides renal nephron segment-specific gene expression.

1. Introduction

Adeno-associated viral (AAV) vectors have become one of the most attractive tools for *in vivo* gene transfer in translational, as well as basic research studies [1]. AAV as a wild-type virus or as a gene-transfer vector does not cause any disease in humans. Recent advances in AAV vector production methods offer preparation of clinical grade AAV vectors, consistently and cost effectively [2–4]. AAV vectors provide sustained long-term gene expression with minimal immunological consequences [5,6]. Systemic administration of AAV9 vector transduces various organs such as liver, heart, lung and skeletal muscle efficiently but provides very low transduction in kidney [6–11].

Several AAV serotypes and routes of administration in combination with kidney specific promoter have been attempted to improve AAV vector-directed gene transfer to the kidney. Systemic administration of AAV1 carrying G6PAse-α provided gene expression in hepatocytes and kidneys that alleviated metabolic abnormalities in a mouse model of type 1α glycogen storage disease [12]. AAV9 carrying kidney-specific cadherin (KSPC) promoter expressing hepatocyte growth factor provided gene expression in the mouse kidney and liver after systemic administration in COL4A3-deficient mice, which attenuated tubulointerstitial fibrosis and repressed fibrotic markers [13]. In both studies, in spite of low renal transduction by AAV9 or AAV1 vector, the therapeutic benefits were achieved largely due to superior hepatic gene expression that acted in an endocrine fashion on the kidney. Subsequently, Ito et al., 2008 [14] demonstrated that the intra pelvic injection of AAV2 vector provides expression predominantly in the renal medulla. To improve the transduction in kidney epithelial cells Chung et al., 2011 [15] administered AAV vectors by retrograde ureteral infusion and showed that AAV8 and AAV9 transduce kidney cells efficiently when compared with other AAV serotypes tested. In previous studies we analyzed the magnitude and distribution of gene

* Corresponding author. The George Washington University, Department of Medicine, 2300 Eye St NW, Ross Hall, Washington, DC 20037, USA.
E-mail address: prk@gwu.edu (P.R. Konkalmatt).

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expression to show that administration of AAV9 vector by retrograde infusion via the ureter provides efficient gene transfer to renal proximal tubule (PT), distal convoluted tubule, and collecting duct (CD) cells with minimum off-target gene expression [11]. Within the kidney, AAV9 administered by retrograde infusion via the ureter provided efficient gene transfer to renal cells including PT, distal convoluted tubule, collecting duct cells, and podocytes [11]. Moreover, retrograde infusion via the ureter circumvented the extra-renal gene expression by AAV9 vector in spite of bearing the ubiquitously active CMV promoter. The renal nephron consists of several segments, each with distinct function to orchestrate overall fluid and electrolyte balance [16]. The distinct function of each nephron segment is attributed to the unique gene expression profiles exhibited by the epithelial cells in the specific nephron segments. Therefore, it is important to express the gene of interest in the desired cell type within the kidney, so that the renal functional parameters measured will represent the function of the gene expressed in the desired cell type within kidney. We hypothesized that use of the promoter of the gene that is expressed exclusively in the desired nephron segment such as, KSPC [17], SGLT2 [18], NKCC2 [19], and E-CAD [20], provides preferential gene expression in entire nephron, PT, TALH, and CD, respectively.

In this study we generated AAV vectors encoding eGFP gene under the control of nephron segment specific promoters KSPC, SGLT2, NKCC2, or ECAD and determined the distribution of gene expression within the kidney following retrograde ureteral infusion of AAV9 vector carrying the promoter constructs.

2. Methods

AAV vectors: The AAV vector pAAVCMVeGFP, harboring the cytomegalovirus (CMV) promoter driving the expression of eGFP [11] was used to generate the sets of AAV vectors carrying kidney-specific promoters encoding eGFP (Fig. 1). The kidney specific promoters were PCR-amplified from the mouse genomic DNA using the primers (Table 1) with appropriate restriction sites at the 5′ and 3'ends:1.3 kb KSPC promoter (GeneBank: AF118228.1) with NotI and HindIII; 1.1 kb SGLT2 promoter (GeneBank: AJ292928.1) with BsrgI and KpnI; 469 bp NKCC2 promoter (GeneBank: U45313.1) with XbaI and HindIII; and 1.25 kb ECAD promoter (GeneBank: AY566874.1) with XbaI and HindIII restriction sites at 5′ and 3′ ends respectively. The PCR amplicons were then inserted in place of CMV promoter in pAAVCMVeGFP using standard molecular biology protocols to create the plasmids pAAVKSPCeGFP, pAAVAGLT2eGFP pAAVNKCC2eGFP or pAAVECADeGFP (Fig. 1). The 1.1 kb SGLT2 promoter is similar to the one described before [18], except that it contains only 400 bp of the 5′ untranslated region and includes an HNF1a binding site, crucial for the transcription form the SGLT2 promoter [21]; the remaining sequence includes the first exon, first intron, and part of the second exon with the starting codon ATG present in the first exon, mutated to AGG to inactivate the gene expression from the endogenous starting codon. Reporter gene expression from each of the promoters was confirmed by transient transfection into HEK 293 cells before proceeding to packaging into AAV9 for *in vivo* studies. Recombinant AAV vector genomes were packaged into AAV9 capsids by triple transfection method in HEK 293 cells [22,23]. AAV vectors were purified by ammonium sulfate

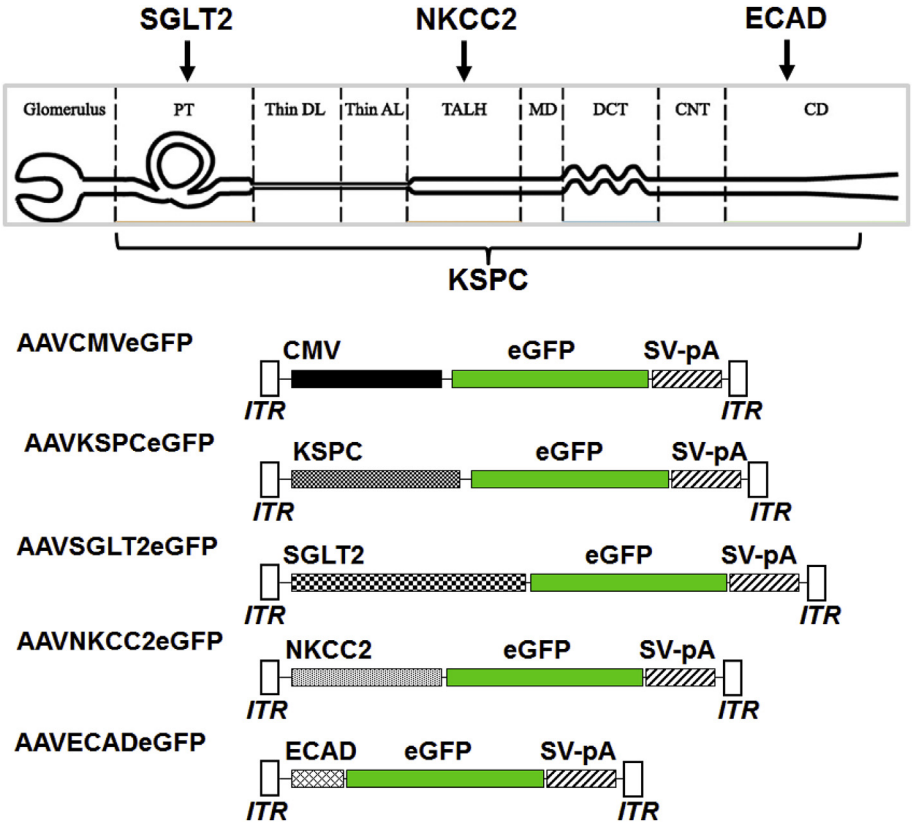

Fig. 1. Diagrammatic representation of the segments of the nephron modified from Nguyen et al., 2012 [42] and choice of promoters for segment-specific gene expression and AAV vector promoter constructs. PT = proximal tubule, DL = descending limb of the loop of Henle, AL = ascending limb of the loop of Henle, TALH = thick ascending limb of the loop of Henle, MD = macula densa, DCT = distal convoluted tubule, CNT = connecting tubule, CD = collecting duct, CMV = cytomegalovirus, eGFP = enhanced green fluorescent protein, SV-pA = Simian virus 40 polyadenylation signal sequence, ITR = inverted terminal repeat sequence, KSPC = kidney-specific cadherin, SGLT2 = sodium-glucose cotransporter 2, NKCC2 = sodium potassium 2-chloride cotransporter, ECAD = e cadherin.