Anellovectors: a novel functional gene delivery platform based on commensal human anelloviruses demonstrates transduction in multiple cell and tissue types

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Abstract

Anelloviridae is a highly diverse family of non-enveloped negative-sense ssDNA viruses that infect vertebrates. While anelloviruses are a ubiquitous component of the human virome, they evade induction of humoral immune response and appear to be apathogenic in humans. These properties make anelloviruses an attractive candidate for harnessing the human virome for the next generation of genetic medicines. Here we report the development of a completely novel gene therapy class termed Anellovectors. Vectorization of anelloviruses is enabled through development of the Self-Amplifying Trans-complementation of a Universal Recombinant Anellovector (SATURN) production system, which relies on a self-replicating plasmid to provide viral proteins in trans that drive replication and packaging of vector genomes. Using the SATURN system, capsid protein-dependent particles that encapsidate ssDNA vector genomes are produced. Like most mature viral vector systems, the vector genome is devoid of viral genes and only carries therapeutic or reporter transgenes. We validated these particles by isopycnic centrifugation, DNase-protected qPCR, next generation sequencing, and electron microscopy. Furthermore, we demonstrate packaging of a vector genome from a single anellovirus with capsids from multiple anellovirus species, suggesting we can build a universal vector platform that takes advantage of the remarkable diversity of anelloviruses. In vitro transduction was validated by detection of an eGFP reporter and detection of vector genomes in nuclei by in situ hybridization. The expression of an eGFP reporter was validated in mouse studies in a variety of tissues using different routes of administration. To our knowledge, this is the first report of a functional anellovirus-based gene therapy vector. Anellovectors have the potential to deliver safe, redosable, and potent therapeutics, helping to expand the reach of programmable medicines.

SATURN production platform yields Anellovectors that are capsid dependent

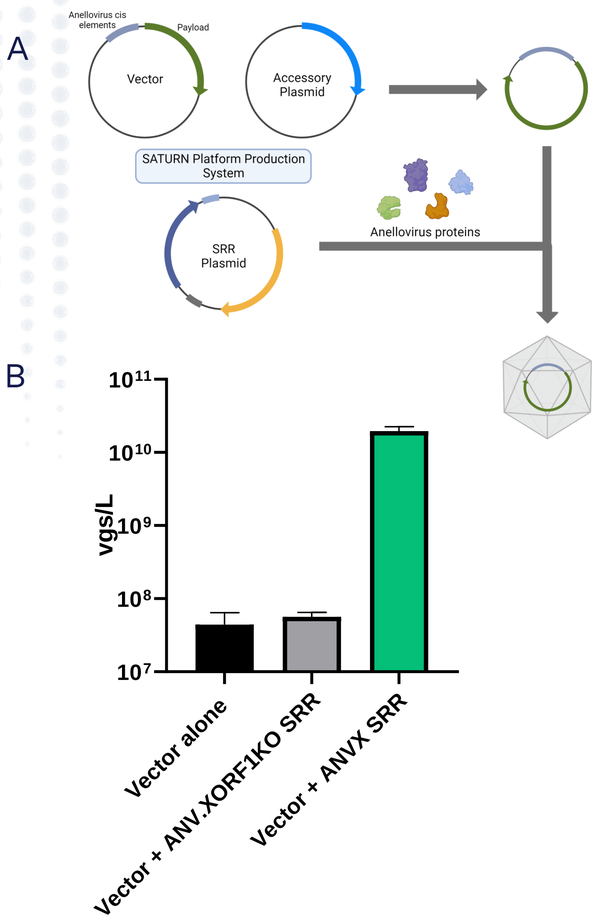

Figure 1. SATURN production platform yields Anellovectors that are capsid dependent. A) Schematic cartoon for the Self-Amplifying Trans-complementation of a Universal Recombinant Anellovector (SATURN) production platform. A vector containing the cis elements required for DNA replication and packaging from anellovirus ANV.X (ANV.X) is transfected along with an accessory plasmid and a self-replicating rescue plasmid (SRR) which provides the required anelloviral proteins for vector DNA replication and packaging in trans. B) A ANV.X-eGFP vector was transfected alone, with a ANV.X-SRR containing a stop codon in ORF1, the capsid gene (ANV.XORF1KO), and accessory plasmid, or with the ANV.X-SRR and accessory plasmid. Vectors were recovered using isopycnic centrifugation, and total DNase-protected eGFP vector DNA was quantified using qPCR and reported as viral genomes (vg) per liter of starting production culture.

Anellovectors package single-stranded DNA into vector particles

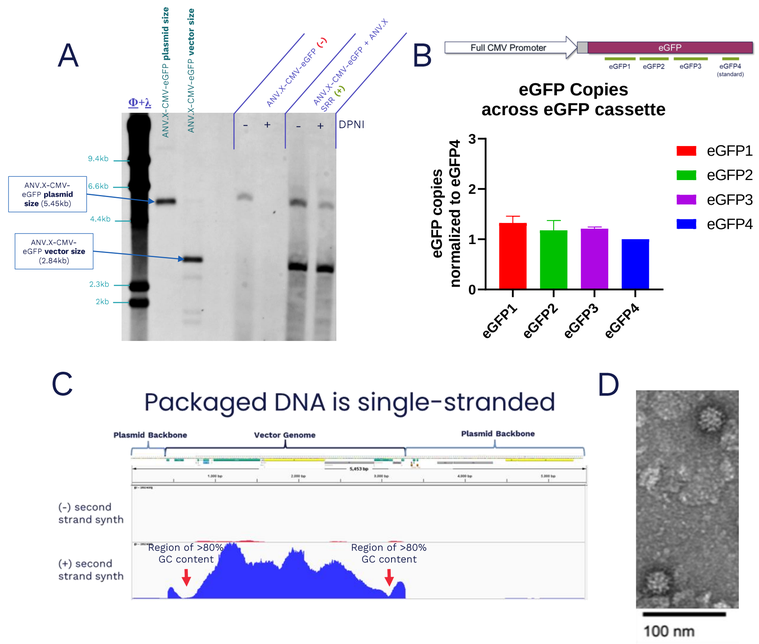

Figure 2. Anellovectors produced with the SATURN production system package ssDNA into vector particles. A) DNA recovered from a SATURN production run was analyzed using a Southern blot. DNA was subjected to DPNI digestion, a restriction enzyme that requires bacterial methylation patterns for activity at its recognition site, before being run on a gel. The membrane was probed for the eGFP sequence. B) ANV.X-eGFP vectors recovered using isopycnic centrifugation were analyzed for total DNase-protected eGFP vector DNA by qPCR using a set of tiled probes against the eGFP gene. C) Recovered ANV.X-eGFP vector was sequenced +/- second strand synthesis and mapped to the vector plasmid. D) An electron micrograph of recovered ANV.X-eGFP vector demonstrating particles of 30nm size.

SATURN is a modular Anellovector platform

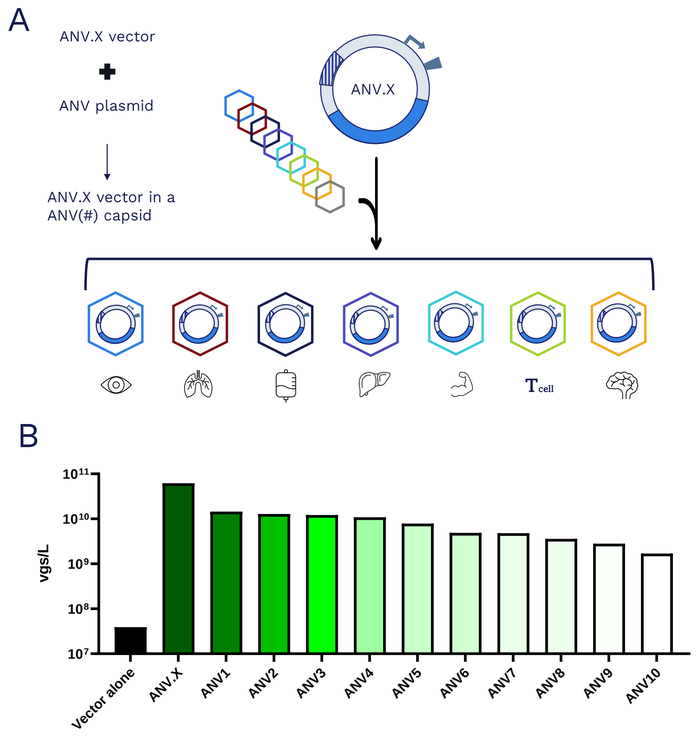

Figure 3. The SATURN production platform is modular. A) A schematic cartoon for using a universal vector genome (here based on ANV.X), which can be encapsidated by proteins from many different anelloviruses derived from a diverse range of tissues using SRRs. B) Vector recovered from SATURN platform production runs using a ANV.X-eGFP vector plasmid and SRRs from various anelloviruses (ANV) were analyzed and compared by total DNase-protected eGFP vector DNA was quantified using qPCR and reported as viral genomes (vg) per liter of starting production culture.

Anellovirus DNA is detectable in the nucleus of RPE cells

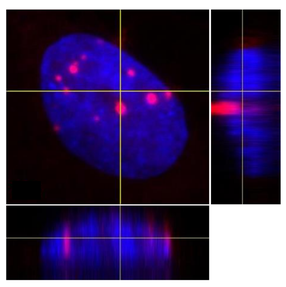

Figure 4. Detection of anellovirus DNA in the nuclei of retinal pigment epithelium (RPE) cells in vitro. RPE cells were transduced with a ANV.X anellovirus at an MOI of 310 vgs/cell. Cells were fixed at 7 hours post transduction. Viral DNA was probed using an DNA-scope protocol against the negative sense input viral genome (red channel; DAPI in blue channel). Confocal images were captured using a 63X objective.

Anellovector demonstrates similar in vivo activity to AAV9 in the mouse brain

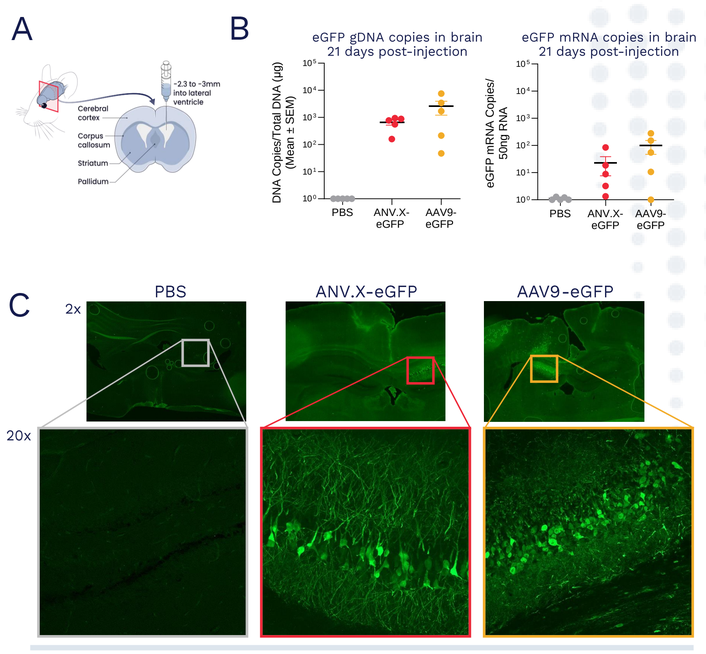

Figure 5. ANV.X-eGFP vector demonstrates CNS transduction comparable to AAV9 in a mouse model. A) Schematic for intracerebroventricular route of administration. On Day 0, FVB mice were injected with either PBS, ANV.X-eGFP, or AAV9-eGFP into the lateral ventricle. B) Tissues were harvested on Day 21 post injection. Total eGFP DNA was quantified using ddPCR, and mRNA was quantified by RT-ddPCR. C) Immunohistochemistry using an antibody against eGFP was used to detect eGFP protein in murine brain tissue of Day 21 samples.

Pilot NHP study shows retinal Anellovector transduction following intravitreal administration

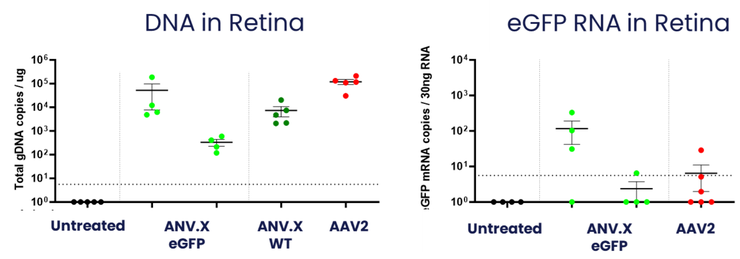

Figure 6. ANV.X-eGFP vector demonstrates retinal transduction in African green monkeys following intravitreal administration. African green monkeys were injected intravitreally with either ANV.X-eGFP, ANV.X-WT or AAV9-eGFP. Retinas were harvested on day 28 post-injection. Total eGFP DNA, or WT ANV.X DNA, was quantified using ddPCR and eGPF mRNA was quantified by RT-ddPCR.

Conclusions

- Tissue specificity, immune evasion, redosability, and lack of integration are unique attributes that make vectorizing anelloviruses appealing and compelling as a viral vector
- The SATURN Anellovector production platform produces vectors that are:
  - Capsid-dependent
  - Package the expected ssDNA vector genomes
  - Produce the expected 30nm particles
- The SATURN production platform is modular and a allows for vectorizing a diverse range of anelloviruses derived from tissues of interest
- Anellovectors have demonstrated comparable in vivo activity to AAV9 when delivered by ICV to the lateral ventricle of mouse brains
- Pilot NHP study represents an important step in demonstrating Anellovector function in primates