a bio-techne brand

Tissue biodistribution of AAV-based gene therapy with the RNAscope® assay

Courtney Anderson, Nidhi Vashistha, Mansi Garg, Xiao-Jun Ma, Chris Bunker, Ruby Hsu
Advanced Cell Diagnostics Inc., 7707 Gateway Blvd, Newark, CA, USA 94560

Highlights

AAV tissue biodistribution and cell tropism are essential pieces of information for every gene therapy development program. The RNAscope® ISH assay is a highly informative method for quantitative, cell-specific measurement of AAV vector and transgene expression. The RNAscope® assay can provide single-cell biodistribution data for every tissue type. In this report we demonstrated the use of the RNAscope® assay to determine therapeutic AAV vector cell tropism and transgene expression in non-human primate retina. We demonstrate the ability of the RNAscope® assay to:

- Visualize AAV vector tissue distribution and transgene expression in retina
- Simultaneously detect vector DNA and transgene RNA
- Identify specific cell populations with a multiplex assay using cell markers and AAV vector probes

Gene therapy is a promising, re-emergent strategy to treat many rare diseases that result from single gene defects. Adeno-associated virus (AAV) has proven to be an effective delivery vehicle with high transduction efficiency, stable transgene expression, broad tropism, and the ability to achieve systemic delivery, making it a safe and efficient gene delivery method for non-dividing cells[1]. However, it is essential to understand the biodistribution of a gene therapy product in target and non-target tissues, as well as the persistence of transgene expression in those tissues. This is critical information prior to advancement into the clinical and IND filing.

The highly specific and single-molecule sensitivity of the RNAscope® ISH assay for the detection of AAV DNA and transgene RNA is an ideal solution to address questions about therapeutic gene delivery vectors (both viral and non-viral) and assess tissue-based transgene expression. The RNAscope® assay detects mRNA and viral vector DNA sequences in frozen and formalin-fixed paraffin-embedded (FFPE) cells and tissues by utilizing a unique probe design and signal amplification strategy that enables visualizing individual RNA molecules as discrete dots[2]. The RNAscope® assay provides an unparalleled sensitive and specific method for cell and tissue-specific assessment of gene therapy vector and transgene expression analysis in any tissue.

In this report we utilized the RNAscope® assay to detect AAV-transduced cells in the monkey retina. A proprietary AAV expression vector driving GFP transgene expression under the control of the CB promoter was injected subretinally into the eyes of Cynomolgus monkeys. RNAscope® probes were

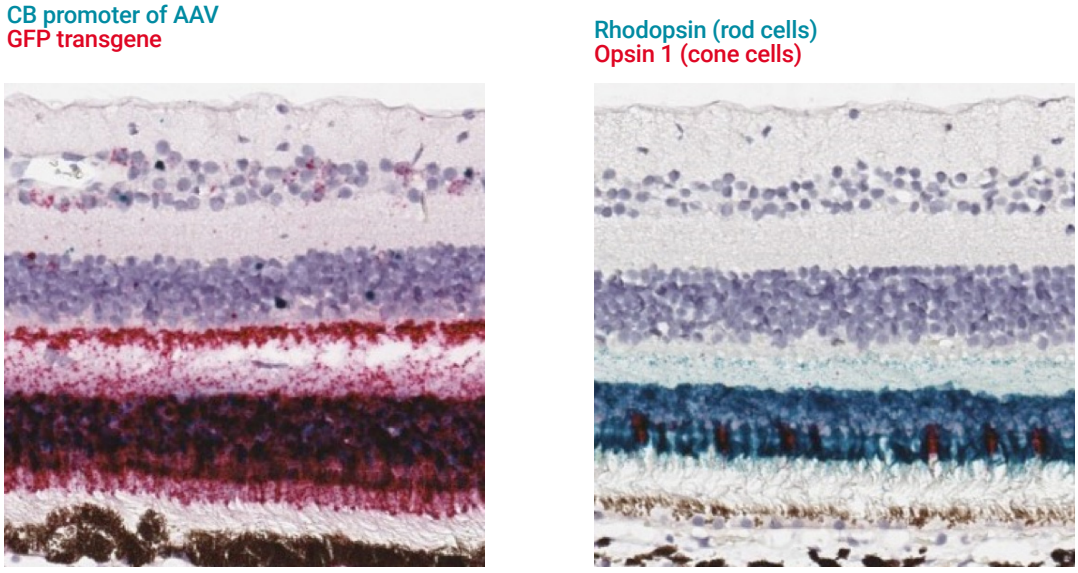

FIGURE 1. Visualization of AAV-transduced cells and specific cell types in the monkey retina. (A) The AAV vector used in this study contains a GFP transgene under the control of the CB promoter. RNAscope® probes were designed to target the CB promoter DNA sequence (V-CBpromoter, cat. no. 423748; green) as well as the GFP transgene mRNA (GFP-C2, cat. no. 409018-C2; red). (B) RNAscope® probes were used to detect the rod cells marked by Rhodopsin (Mfa-RHO, cat. no. 503768; green) and cone cells marked by Opsin 1 short wave sensitive (Mfa-OPN1SW-C2, cat. no. 503778-C2; red).

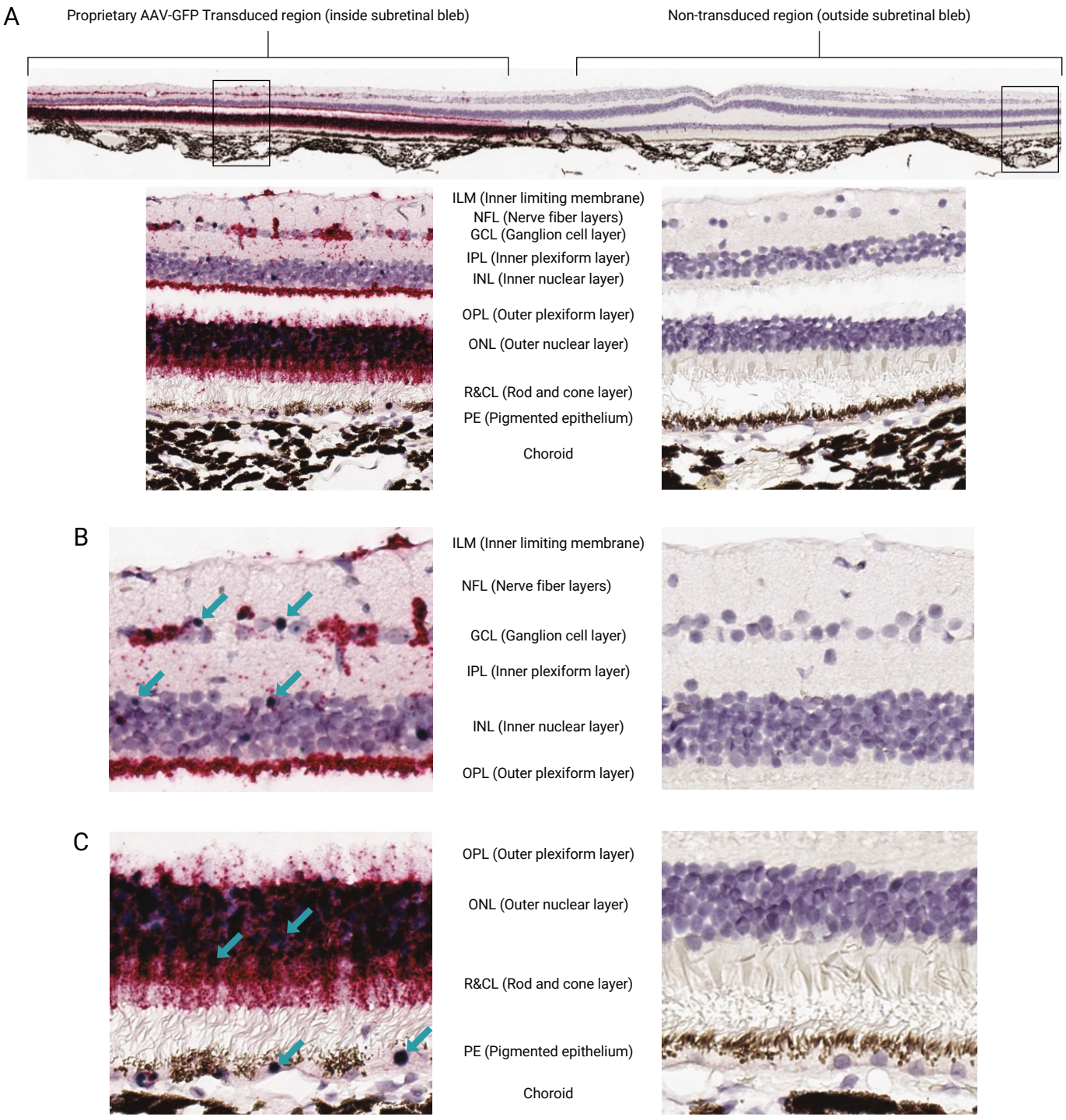

FIGURE 2. Identification of specific cells transduced by AAV-GFP following subretinal injection. (A) The RNAscope® 2.5 HD Duplex assay was used to simultaneously detect the CB promoter DNA sequence of the AAV vector (green) and the GFP transgene mRNA (red). Staining was observed in almost all of the retinal layers (with the exception of the choroid) in the transduced region (left), but no staining was observed in the non-transduced region (right). Green arrows denote AAV-containing cells. (B, C) Higher magnification of the upper (B) and lower (C) layers of the retina.

designed to target the CB promoter DNA sequence of the AAV vector and the GFP transgene mRNA, as well as the specific cell-type markers Rhodopsin (a marker of rod photoreceptor cells) and Opsin 1 (a marker of cone photoreceptor cells) (Figure 1). Figure 2 shows an overview of the retina probed simultaneously for the AAV vector and GFP transgene, with a distinct AAV-GFP transduced region inside the subretinal bleb and a non-transduced region outside the subretinal bleb. In the transduced region the AAV vector was detected primarily in the nucleus of cells in almost all retinal layers, while robust GFP transgene expression was detected in both the nucleus and cytoplasm in almost all retinal layers. Neither the AAV vector nor the GFP transgene was detected in the non-

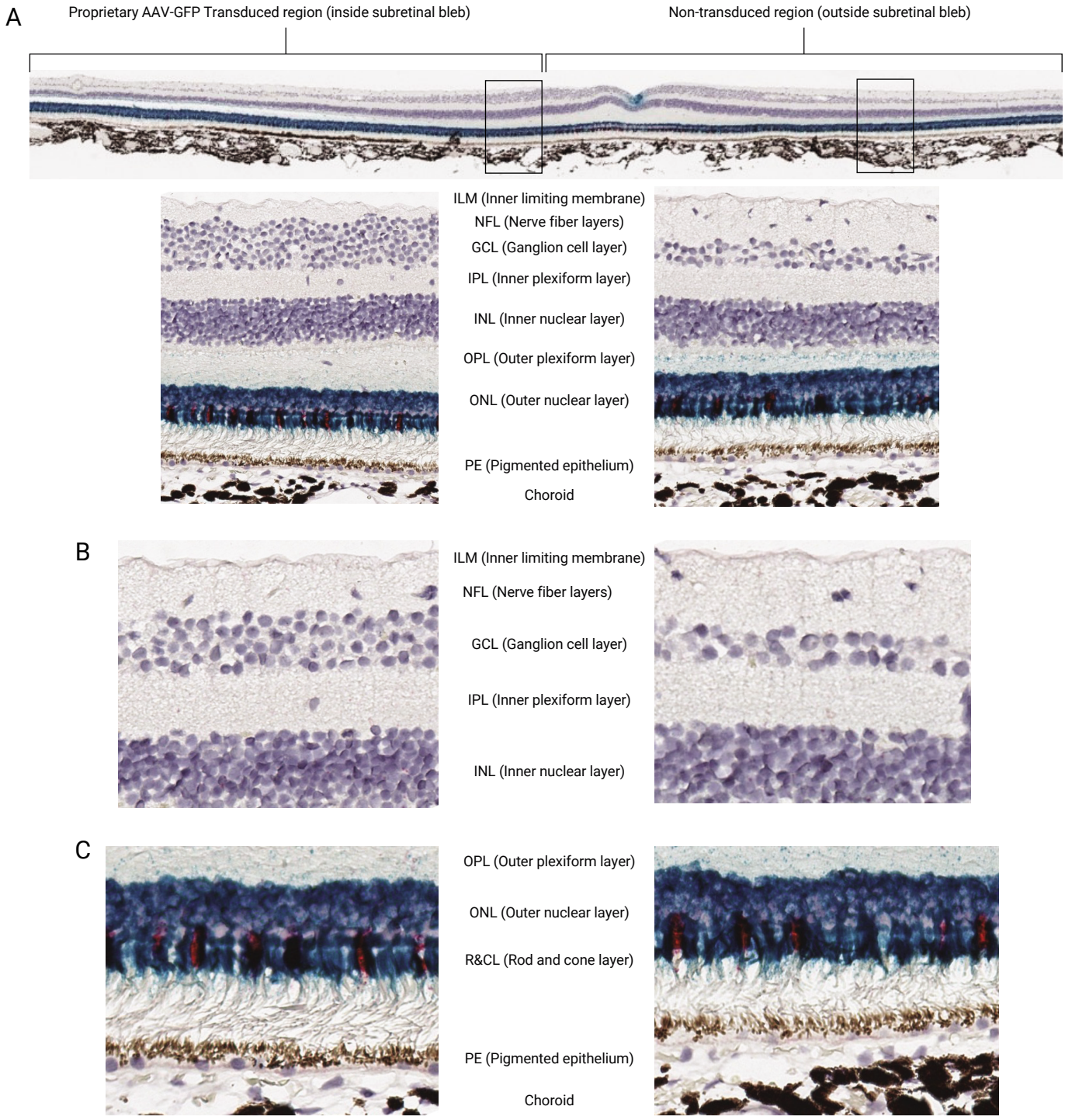

FIGURE 3. Visualization of rod and cone cells in the monkey retina. The RNAscope® 2.5 HD Duplex assay was used to simultaneously detect rod cells marked by Rhodopsin (green) and cone cells marked by Opsin 1 (red). Staining was observed only in the rod and cone cell layer in both the transduced (left) and non-transduced (right) regions. (B, C) Higher magnification of the upper (B) and lower (C) layers of the retina.

transduced region. Specific retinal cell populations were identified using probes for Rhodopsin and Opsin 1, which detected rod cells and cone cells, respectively, in both the transduced and non-transduced regions (Figure 3). Sample RNA quality was confirmed using positive and negative controls (Figure 4).

Conclusions

Tissue biodistribution is critical information in preclinical gene therapy development. Likewise, confirmation of persistent expression of a codon-optimized therapeutic transgene in targeted cells is equally important. In this study we demonstrate the ability of the RNAscope® assay to accurately identify the specific cells

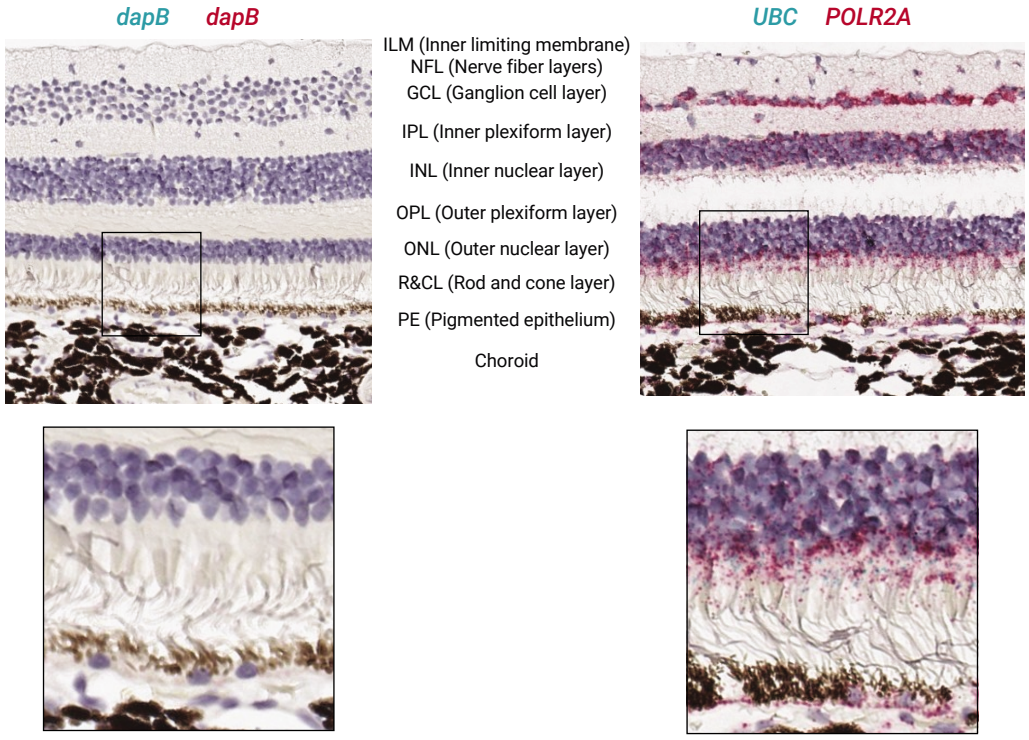

FIGURE 4. Sample quality and assay technical controls. The RNAscope® 2.5 HD Duplex assay detected the positive controls *UBC* (green) and *POLR2A* (red) in all retinal layers and in both the transduced and non-transduced regions. Staining with the negative control probe dapB did not detect any signal in the entire retina.

transduced by AAV in the monkey retina. Cell-specific marker expression was observed in both the non-transduced and proprietary AAV-transduced regions: Rhodopsin was expressed in the rod photoreceptor cells and Opsin 1 was expressed in the cone photoreceptor cells. The RNAscope® assay showed that the proprietary AAV vector targeted the photoreceptor and additional retinal cells and that the vector transgene (GFP) was expressed in the transduced region and not detected in the non-transduced region (outside the subretinal bleb). Overall, the RNAscope® technology enables visualization and quantification of viral DNA copy number and transgene expression with cell-specific localization in intact, fixed tissue. For more information please visit us online at www.acdbio.com/genetherapy.

References

1. Hastie E and Samulski RJ. Adeno-associated virus at 50: A golden anniversary of discovery, research, and gene therapy success — a personal perspective. *Hum Gene Ther.* 2015; 26:257–265.
2. Wang F, et al. RNAscope®: A novel *in situ* RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *J Mol Diagn.* 2012; 14(1):22–9.
3. Polinski NK, et al. Impact of age and vector construct on striatal and nigral transgene expression. *Mol Ther Methods Clin Dev*. 2016; 3, 16082.
4. Keeler AM, et al. Cellular analysis of silencing the Huntington's Disease gene using AAV9 mediated delivery of artificial micro RNA into the striatum of Q140/Q140 mice. *J Huntingtons Dis.* 2016; 5(3): 239–248.
5. Borel F, et al. Therapeutic rAAVrh10 mediated SOD1 silencing in adult SOD1G93A mice and nonhuman primates. *Hum Gene Ther.* 2016; 27(1):19–31.
6. Golebiowski D, et al. Direct intracranial injection of AAVrh8 encoding B-N-acetylhexosaminidase causes neurotoxicity in primate brain. *Hum Gene Ther.* 2017; 28(6): 510–522.

Learn more about RNAscope® technology for gene therapy at
www.acdbio.com/genetherapy

a bio-techne brand

California, USA