Published in final edited form as:

Graefes Arch Clin Exp Ophthalmol. 2013 April ; 251(4): 1245–1246. doi:10.1007/s00417-012-2103-8.

CD44 Isoforms in Human Retinal and Choroidal Endothelial Cells

Timothy J. Chipps, MAT¹, Binoy Appukuttan, PhD¹, Yuzhen Pan, MB¹, and Justine R. Smith, FRANZCO, PhD^{1,2,†}

¹Casey Eye Institute, Oregon Health & Science University, Portland, Oregon, USA

²Department of Cell and Developmental Biology, Oregon Health & Science University, Portland, Oregon, USA

Keywords

CD44; human; retina; choroid; endothelial cell

Dear Editor,

CD44 is a widely expressed type I transmembrane glycoprotein that functions as the receptor for hyaluronic acid, as well as multiple other ligands.¹ In humans, the extracellular domain of “standard isoform” CD44 is encoded by 7 invariant exons (i.e., exons 1 – 5 and exons 15 – 16), but 9 additional alternatively spliced exons (i.e., exons 6 – 14) code for numerous “variant isoforms”.^{2, 3} Exon 17 encodes a transmembrane segment, and exons 18 or 19 encode a cytoplasmic domain. Although the function of specific variant isoforms is still a subject of investigation, it is clear that differences in CD44 structure translate to differences in function.¹

Studies in experimental models have implicated retinal endothelial CD44 in leukocyte trafficking in posterior uveitis,⁴ and choroidal endothelial CD44 in the neovascularization that characterizes wet age-related macular degeneration (AMD).⁵ Human retinal and choroidal endothelial cells express CD44.⁶ Surprisingly, however, the CD44 isoform(s) present in these cell populations are unknown. Using standard RT-PCR and dye-terminator sequencing, and human primary ocular endothelial cell isolates, we confirmed the presence of standard CD44 and investigated the potential expression of CD44 variants in human retinal and choroidal endothelial cells.

Retinal and choroidal endothelial cells were isolated from paired eyes of 5 human cadaver donors with no history of ocular pathology, according to our previously described method.⁶ In brief, isolation involved type II collagenase (Sigma-Aldrich, St. Louis, MO) and Dispase (GIBCO-Invitrogen, Grand Island, NY) digestion of dissected tissue, followed by endothelial purification using magnetic Dynabeads (Dynal-Invitrogen, Brown Deer, WI) conjugated to murine anti-human CD31 antibody (BD Pharmingen, San Diego, CA). Timing was as short as practical to minimize processing-related gene expression changes; isolation was commenced 14 to 19 hours after death, and cells were used in experiments at passage 2 to 5. Total RNA was prepared from confluent endothelial cultures, using the RNeasy Mini Kit (Qiagen, Valencia, CA) with optional on-column DNase treatment, and cDNA was

[†]Corresponding Author: Justine R. Smith, FRANZCO, PhD, Address: Oregon Health & Science University, Mail Code: L467AD, Biomedical Research Building, 3181 S.W. Sam Jackson Park Rd., Portland, OR, USA 97239, Telephone: (503) 494-5023, Facsimile: (503) 494-6875, smithjus@ohsu.edu.

The authors have no financial relationship with the organizations that sponsored the research (see Acknowledgements). The authors have full control of all primary data.

synthesized using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). The PCR was performed on a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Carlsbad, CA), using published primers designed to recognize sequence in invariant CD44 exons 4 (forward: 5'-ACATCAGTCACAGACCTGCC-3') and 17 (reverse: 5'-GCAAAGTGAAGAATCAAAGCC-3'),³ as well as GAPDH (5'-AGCTGAACGGGAAGCTCACTGG-3' and 5'-GGAGTGGGTGTCGCTGTGAAGTC-3').⁶ Additional PCRs were performed using forward primers for variant CD44 exons 6 (5'-CCTGGGATTGGTTTTCATGGTT-3'), 8 (5'-AACCAGGACTGGACCCAGTGGAA-3'), 10 (5'-AGGAACAGTGGTTTGGCAAC-3') and 12 (5'-GACAGGACCTCTTTCAATGAC-3'), designed in-house, and the reverse primer for invariant exon 17. All CD44 PCR products were purified with QIAquick Gel Extraction kit (Qiagen, Valencia, CA) and sequenced on the ABI 3130x/Genetic Analyzer (Applied Biosystems).

By RT-PCR using primers located at either side of the variant exon sequence in the extracellular domain of CD44, we confirmed the expression of standard CD44 in retinal and choroidal endothelial cells from the five human donors. In addition, with forward primers located within the variant exon sequence and a reverse primer in conserved sequence, we detected 10 alternatively spliced transcripts containing one or more of the 9 variant exons, although interestingly, choroidal endothelial cells from some donors did not express all isoforms (Table 1).

Our work demonstrates that, not unexpectedly, human retinal and choroidal endothelial cells express standard CD44. However, and importantly, multiple variant CD44 isoforms are synthesized by these cell types, although not universally. We identified 10 variant isoforms in both ocular endothelial cell populations, with all variant exons represented, and we anticipate additional isoforms would be detected by other methods. CD44 has been identified as a potential therapeutic target in ocular vascular disease.⁵ Given that the function of CD44 may vary, depending on the isoform, our findings have implications for further studies in this area. In such work, it would be critical to identify the specific CD44 isoform(s) involved in any pathological process(es) responsible for the disease in question. The potential for donor-specific splicing events should be an additional consideration.

Acknowledgments

This work was supported by grants from the National Institutes of Health (RO1 EY019875 and RO1 EY019042), the International Retina Research Foundation, and Research to Prevent Blindness (unrestricted grant to Casey Eye Institute).

References

1. Bajorath J. Molecular organization, structural features, and ligand binding characteristics of CD44, a highly variable cell surface glycoprotein with multiple functions. *Proteins*. 2000; 39:103–111. [PubMed: 10737932]
2. Sreter GR, Bell MV, Jackson DG, Cornelis FB, Gerth U, Bell JI. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. *Proc Natl Acad Sci U S A*. 1992; 89:12160–12164. [PubMed: 1465456]
3. Liu NP, Roberts WL, Hale LP, Levesque MC, Patel DD, Lu CL, Jaffe GJ. Expression of CD44 and variant isoforms in cultured human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci*. 1997; 38:2027–2737. [PubMed: 9331266]
4. Xu H, Manivannan A, Jiang HR, Liversidge J, Sharp PF, Forrester JV, Crane IJ. Recruitment of IFN-gamma-producing (Th1-like) cells into the inflamed retina in vivo is preferentially regulated by P-selectin glycoprotein ligand 1:P/E-selectin interactions. *J Immunol*. 2004; 172:3215–2324. [PubMed: 14978129]

5. Mochimaru H, Takahashi E, Tsukamoto N, Miyazaki J, Yaguchi T, Koto T, Kurihara T, Noda K, Ozawa Y, Ishimoto T, Kawakami Y, Tanihara H, Saya H, Ishida S, Tsubota K. Involvement of hyaluronan and its receptor CD44 with choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2009; 50:4410–4415. [PubMed: 19339747]
6. Smith JR, Choi D, Chipps TJ, Pan Y, Zamora DO, Davies MH, Babra B, Powers MR, Planck SR, Rosenbaum JT. Unique gene expression profiles of donor-matched human retinal and choroidal vascular endothelial cells. *Invest Ophthalmol Vis Sci*. 2007; 48:2676–2684. [PubMed: 17525199]

Table 1

Exonic sequence of the variable segment of the extracellular domain in CD44 variant isoforms. Nine alternatively spliced exons (i.e., exons 6 – 14) encode for variants. Results are for human retinal and choroidal endothelial isolates from 5 human cadavers.

Exonic sequence of variable segment of extracellular domain	Endothelial samples	
	Retinal (n=5)	Choroidal (n=5)
ex6	5	5
ex6–ex7	5	5
ex8	5	3
ex8–ex9	5	3
ex10	5	5
ex10–ex11	5	5
ex10–ex11–ex12–ex13–ex14	5	5
ex12	5	4
ex12–ex13	5	4
ex12–ex13–ex14	5	4

Abbreviations: ex=exon