Genomics 105 (2015) 253–264

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Review

Transcriptome of the human retina, retinal pigmented epithelium and choroid

Lifeng Tian [a], Krista L. Kazmierkiewicz [b], Anita S. Bowman [b], Mingyao Li [c], Christine A. Curcio [d], Dwight E. Stambolian [b,*]

[a] Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, Pa 19104, USA
[b] Department of Ophthalmology, University of Pennsylvania, Philadelphia, Pa 19104, USA
[c] Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, Pa 19104, USA
[d] Department of Ophthalmology, University of Alabama School of Medicine, Birmingham, Al 35294, USA

ARTICLE INFO

Article history:
Received 28 August 2014
Accepted 24 January 2015
Available online 31 January 2015

Keywords:
Age-related macular degeneration
cDNA microarray
Glaucoma
Retina
Retinal pigmented epithelium
Choroid
RNA-Seq
Serial analysis of gene expression
Transcriptome

ABSTRACT

The retina and its adjacent supporting tissues – retinal pigmented epithelium (RPE) and choroid – are critical structures in human eyes required for normal visual perception. Abnormal changes in these layers have been implicated in diseases such as age-related macular degeneration and glaucoma. With the advent of high-throughput methods, such as serial analysis of gene expression, cDNA microarray, and RNA sequencing, there is unprecedented opportunity to facilitate our understanding of the normal retina, RPE, and choroid. This information can be used to identify dysfunction in age-related macular degeneration and glaucoma. In this review, we describe the current status in our understanding of these transcriptomes through the use of high-throughput techniques.

Contents

1. Introduction . . . 254
2. Structure of the human eye: retina and choroid . . . 254
3. Transcriptome analysis of the retina and RPE/choroid . . . 254
   3.1. High-throughput technologies: cDNA microarray, SAGE and RNA-Seq . . . 254
   3.2. RNA qualities and methods of case ascertainment . . . 255
4. Exploring the human retina transcriptome . . . 257
   4.1. Number of genes detected in retina . . . 257
   4.2. Effect of aging on gene expression . . . 257
   4.3. Effect of retina anatomic location on gene expression . . . 258
   4.4. RNA-Seq assesses alternative splicing . . . 258
   4.5. Comparison between RNA-Seq and Microarray . . . 258
5. Exploring the human RPE/choroid transcriptome . . . 258
   5.1. Number of genes detectable by microarray . . . 258
   5.2. Effect of anatomic location on gene expression . . . 259

Abbreviations: AMD, age-related macular degeneration; eQTL, expression quantitative trait loci; GWAS, genome-wide association studies; FPKM, Fragments Per Kilobase of gene per Million mapped fragment; MAQC, MicroArray Quality Control; MR, macular retina; PLIER, Probe Logarithmic Intensity Error; PR, peripheral retina; RPE, retinal pigment epithelium; RNA-Seq, RNA sequencing; SAGE, serial analysis of gene expression.

* Corresponding author at: 313B Stellar-Chance Labs, 422 Curie Blvd., Philadelphia, PA 19104, USA. Fax: +1 215 573 6728.
E-mail addresses: tianl@email.chop.edu (L. Tian), kristakazmar@gmail.com (K.L. Kazmierkiewicz), anitabow@mail.med.upenn.edu (A.S. Bowman), mingyao@mail.med.upenn.edu (M. Li), curcio@uab.edu (C.A. Curcio), stamboli@mail.med.upenn.edu (D.E. Stambolian).

http://dx.doi.org/10.1016/j.ygeno.2015.01.008
0888-7543/

6. Transcriptome differences between human retina and RPE/choroid . . . 259
6.1. Comparison of differential expression . . . 259
6.2. Comparison of pathway analysis . . . 260
7. The transcriptome in AMD and glaucoma . . . 260
7.1. Age-related macular degeneration . . . 260
7.2. Glaucoma . . . 261
8. Bioinformatic databases for human retina and RPE/choroid transcriptomes . . . 261
9. Conclusions . . . 262
Competing interests . . . 263
Authors' contributions . . . 263
Acknowledgments . . . 263
References . . . 263

1 . Introduction

In the past decade, multiple common genetic variants have been identified for prevalent diseases through genome-wide association studies (GWAS). Translating the results of GWAS to new treatments for disease still faces several challenges including: 1) Single nucleotide polymorphisms (SNPs) significant in GWAS are not causative and may be in linkage disequilibrium with causative SNPs that are distantly located; 2) associated genes harboring these SNPs have not been evaluated in tissues affected by disease; and 3) gene expression in cells central to the disease have not been evaluated for regulatory effects of these SNPs. While accumulating scientific evidence suggests that regulatory changes contribute to the phenotypes interrogated by GWAS, progress in defining these regulatory changes has been slow. This problem is apparent in the eye, a highly specialized structure composed of multiple layers and cells derived from mesoderm and neuroectoderm that has produced multiple GWAS hits outside coding regions for age-related macular degeneration (AMD), glaucoma, and refractive error, three widely prevalent eye conditions. Here, we focus on AMD and glaucoma, the most prevalent causes of untreatable vision loss in the elderly.

The transcriptome is a complex mixture of multiple isoforms for known genes and non-coding RNAs that will require multiple approaches for characterization. These multiple transcript types are heritable, based on evidence that gene expression is heritable between monozygotic twins [1], Centre d'Etude du Polymorphisme Humain families [2,3], and Icelandic families [4]. The transcriptome is also cell/tissue specific, requiring cell specific characterization. For example, there is lack of significant sharing of cis-expression quantitative trait loci (eQTLs) between adipose tissue and blood [4] or between brain cortical tissue and blood mononuclear cells [5], making it necessary to characterize the transcriptome of each cell type.

Vision scientists have long recognized the importance of characterizing the transcriptome through their use of microarrays to evaluate expression. This technique persists as a mainstay among vision scientists even though microarrays have been shown to have biases in hybridization strength, as well as the potential for cross hybridization to probes with similar sequences [6]. Herein, we provide a brief overview of the eye structure with an emphasis on its posterior layers, and follow with a review of SAGE, cDNA microarray, and RNA sequencing (RNA-Seq) studies of the protein-coding transcriptomes of human retina, retinal pigmented epithelium (RPE) and choroid.

2 . Structure of the human eye: retina and choroid

The retina is a specialized neural tissue lining the back of the eye that is responsible for vision. The retina originates as an outgrowth of the brain during ontogenesis and is thus part of the central nervous system. There are 21 chorioretinal layers and clinically important spaces, many of which can be visualized non-invasively at high-resolution (Fig. 1) [7]. The macula has the highest overall density of neurons and is responsible for acute central vision (diameter = 6 mm = 21° of visual angle). The nasal retina (close to the nose, seeing the temporal visual field) has 1.4–3-fold more neurons, depending on the cell type, than temporal retina (close to the temple, seeing the nasal visual field.) The cellular composition of the neurosensory retina is highly organized with a variety of neurons and two glial types. Major neuron types have been historically classified based on morphology, laminar distribution, topography, brain connections, physiological responses to light stimuli, transmitter pharmacology, and molecular signatures, such as immunoreactivity to specific antibodies. These cells likely differ by their RNA transcriptional programs as well. A recent classification of retinal cell types indicates three cone photoreceptors, one rod photoreceptor, two horizontal cells, 13 bipolar cells, ≥29 amacrine cells, and ≥20 ganglion cells. Only the ganglion cells project to the brain (via the optic nerve). Ganglion cells are preferentially affected in glaucoma, a prevalent cause of vision loss for which high intraocular pressure is an important risk factor. Glial cells important for retinal function are Müller cells that span all the neuronal layers and astrocytes among ganglion cell axons *en route* to the optic nerve head. The RPE and choroidal vasculature constitute the photoreceptor support system, which is affected in AMD, a major cause of vision loss in the elderly of European descent worldwide. Polarized RPE has demanding dual roles serving photoreceptors apically and choroid basolaterally. Distinctive extracellular lesions that differentially confer risk for AMD progression distributes on both aspects of this key cell layer [8–10]. The retina has two vascular beds with different properties and propensity for disease. The retinal circulation serves the inner retina and is within the blood–retina barrier. The choroid serves the photoreceptors and RPE, and it is part of the systemic circulation. The RPE maintains the outer limit of the blood–retina barrier with junctional complexes. The choroid is distinguished by the highest blood flow in the body, especially under the macula, and it thins markedly with aging [11]. Choroidal cells include vascular and lymphatic endothelia, smooth muscle cells, fibroblasts, melanocytes, mast cells, autonomic neuronal ganglia, and resident and transient cells of monocyte lineage.

3 . Transcriptome analysis of the retina and RPE/choroid

3.1. High-throughput technologies: cDNA microarray, SAGE and RNA-Seq

A cDNA microarray consists of immobilized probes complementary to known transcripts on a solid substrate [12]. Isolated RNA is labeled with fluorescent dyes and hybridized to the cDNA microarrays, washed, and scanned with a laser scanner. The amount of fluorescent dye intensity is a measure of gene expression. In early versions of cDNA microarrays, biases and artifacts produced inconsistent results among the same samples. In 2006, quality control standards were developed by the MicroArray Quality Control (MAQC) to address these issues [13]. Still, microarrays are unable to identify RNA editing events or novel isoforms, and they cannot accurately measure absolute expression levels due to hybridization and background variation [14].

Serial analysis of gene expression (SAGE) can perform a quantitative analysis of transcripts without requiring prior knowledge of the