Experimental Eye Research 147 (2016) 57–71

Contents lists available at ScienceDirect

Experimental Eye Research

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

Review

Clusterin in the eye: An old dog with new tricks at the ocular surface

M. Elizabeth Fini [a, *], Aditi Bauskar [b], Shinwu Jeong [c], Mark R. Wilson [d]

[a] USC Institute for Genetic Medicine and Departments of Cell & Neurobiology and Ophthalmology, Keck School of Medicine of USC, University of Southern California, 2250 Alcatraz St., Suite 240, Los Angeles, CA 90089-9037, USA
[b] USC Institute for Genetic Medicine and Graduate Program in Medical Biology, Keck School of Medicine of USC, University of Southern California, 2250 Alcatraz St., Suite 240, Los Angeles, CA 90089-9037, USA
[c] USC Institute for Genetic Medicine and Department of Ophthalmology, Keck School of Medicine of USC, University of Southern California, 2250 Alcatraz St., Suite 240, Los Angeles, CA 90089-9037, USA
[d] Illawarra Health and Medical Research Institute, School of Biological Sciences, University of Wollongong, Northfields Avenue, Wollongong, New South Wales, 2522 Australia

ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form
22 April 2016
Accepted in revised form 23 April 2016
Available online 27 April 2016

Keywords:
Clusterin
Chaperone
Ocular surface
Cornea
Proteostasis
Cytoprotection
Inflammation
Dry eye
Epithelial barrier
Mucosal epithelium
Squamous metaplasia
Biotherapeutic

ABSTRACT

The multifunctional protein clusterin (CLU) was first described in 1983 as a secreted glycoprotein present in ram rete testis fluid that enhanced aggregation ('clustering') of a variety of cells *in vitro*. It was also independently discovered in a number of other systems. By the early 1990s, CLU was known under many names and its expression had been demonstrated throughout the body, including in the eye. Its homeostatic activities in proteostasis, cytoprotection, and anti-inflammation have been well documented, however its roles in health and disease are still not well understood. CLU is prominent at fluid-tissue interfaces, and in 1996 it was demonstrated to be the most highly expressed transcript in the human cornea, the protein product being localized to the apical layers of the mucosal epithelia of the cornea and conjunctiva. CLU protein is also present in human tears. Using a preclinical mouse model for desiccating stress that mimics human dry eye disease, the authors recently demonstrated that CLU prevents and ameliorates ocular surface barrier disruption by a remarkable sealing mechanism dependent on attainment of a critical all-or-none concentration in the tears. When the CLU level drops below the critical all-or-none threshold, the barrier becomes vulnerable to desiccating stress. CLU binds selectively to the ocular surface subjected to desiccating stress *in vivo*, and *in vitro* to LGALS3 (galectin-3), a key barrier component. Positioned in this way, CLU not only physically seals the ocular surface barrier, but it also protects the barrier cells and prevents further damage to barrier structure. CLU depletion from the ocular surface epithelia is seen in a variety of inflammatory conditions in humans and mice that lead to squamous metaplasia and a keratinized epithelium. This suggests that CLU might have a specific role in maintaining mucosal epithelial differentiation, an idea that can now be tested using the mouse model for desiccating stress. Most excitingly, the new findings suggest that CLU could serve as a novel biotherapeutic for dry eye disease.

1. Introduction

The multi-functional protein clusterin was first described in 1983 as a secreted glycoprotein present in ram rete testis fluid that enhanced aggregation ('clustering') of a variety of cells *in vitro* (Fritz et al., 1983; Blaschuk et al., 1983). The protein was subsequently re-identified in a number of other studies and was given different names based on the activity investigated. Clusterin is identical to serum protein 40,40 (SP-40,40) found in the SC5b-complex of complement and in immune deposits in glomerulonephritis (Murphy et al., 1988; Tsuruta et al., 1990). It is also the same as Apolipoprotein J (ApoJ), a protein associated with high-density lipoprotein and very high-density lipoprotein in human serum (de Silva et al., 1990a; James et al., 1991), as well as sulfated glycoprotein-2 (SGP-2), the major secreted product of rat Sertoli cells (Tsuruta et al., 1990), and the protein translated from testosterone-repressed prostate message-2 (TRPM-2), which is

Abbreviations: FDA, U.S. Food & Drug Administration; FECD, Fuchs' Endothelial Corneal Dystrophy; HDL, high-density lipoprotein; PXG, pseudoexfoliation glaucoma; RT-PCR, Reverse Transcriptase-Polymerase Chain Reaction.

* Corresponding author.
E-mail addresses: efini@usc.edu (M.E. Fini), bauskar@usc.edu (A. Bauskar), shinwuje@usc.edu (S. Jeong), mrw@uow.edu.au (M.R. Wilson).

http://dx.doi.org/10.1016/j.exer.2016.04.019
0014-4835/

upregulated in the regressing rat ventral prostate (Leger et al., 1987). Participants in the inaugural International Workshop on Clusterin held in Cambridge, England in 1992 agreed to the name clusterin, acknowledging the original reports of its identification (Wilson and Easterbrook-Smith, 2000). The HUGO nomenclature committee has given clusterin the designation "CLU".

CLU is nearly ubiquitously expressed in tissues, and is constitutively present in most biological fluids (Wyatt et al., 2013a). The first publication on CLU in the eye was in 1992, describing elevated CLU expression in the degenerative disorder, retinitis pigmentosa (Jones et al., 1992). CLU expression in various parts of the eye was subsequently documented in developmental studies in rats (Ahuja et al., 1994) and mice (Reeder et al., 1995), including in the lens, cornea and ciliary body, and CLU protein was demonstrated in the aqueous and vitreous of the mature human eye (Reeder et al., 1995). A number of studies at that time investigated a role for CLU in retinal degenerative disease. In 1996, a DNA sequencing study was published highlighting CLU as the most highly expressed gene in the adult human corneal epithelium (Nishida et al., 1996a), sparking interest in examining the role of CLU at the ocular surface, as discussed below. The most recent study of expression demonstrated CLU mRNA in adult human and monkey eyes localized to the lens, cornea, limbus, sclera, orbital muscle, ciliary body, retina, and retinal pigment epithelium/choroid, as well as to retinal pigment epithelial cells in culture (Wong et al., 2000).

When we began to write this article, we performed a search of PubMed using the term "clusterin", and turned up more than 2000 articles. Despite all this research, new knowledge continues to emerge. We refer the reader to the numerous excellent review and perspective articles on CLU, a selection of which are listed here (Wilson and Easterbrook-Smith, 2000; Rosenberg et al., 1993; Rosenberg and Silkensen, 1995; Koltai, 2014; Jenne and Tschopp, 1992; May and Finch, 1992; Jones and Jomary, 2002; Trougakos and Gonos, 2002; Yerbury et al., 2016). The current article provides a brief overview of the history and current knowledge on CLU. It then offers an updated review and perspective on the physiologic role of CLU in the eye, including some new insight from our group on its role at the ocular surface (Jeong et al., 2012; Bauskar et al., 2015).

2. Gene and protein structure

In humans, a single *CLU* gene of nine exons is located on chromosome 8. The sequence is highly conserved across species, showing 70–80% identity at the amino acid level amongst mammals (Jones and Jomary, 2002). Transcription results in an mRNA of ~2-kb, from which is produced a primary polypeptide chain of 449 amino acids. Fig. 1 is a schematic of the CLU molecule based on information deduced from sequence analysis and biochemical studies. An N-terminal signal peptide of 22 amino acids is removed in the endoplasmic reticulum to produce a protein with a predicted mass of ~50 kDa. Subsequently, CLU is proteolytically cleaved to form two anti-parallel polypeptide chains of similar size connected at a central core by 5 disulfide bonds. Six predicted N-linked glycosylation sites clustered around the disulfide-bonded core were confirmed by mass spectroscopy (Kapron et al., 1997). This results in a secreted glycoprotein with an apparent mass of 75–80 kDa by SDS-PAGE, although the actual mass is approximately 58–63 kDa, which is 17–27% carbohydrate by weight. Other N-terminally truncated clusterin isoforms have been proposed, including one thought to localize to the nucleus (e.g., Trougakos et al. (2009); Leskov et al. (2003)), however unequivocal identification of any of these in cells has yet to be achieved.

Sequence analysis of the CLU mRNA predicts that the glycosylated, disulfide-bonded core of the encoded protein is flanked by

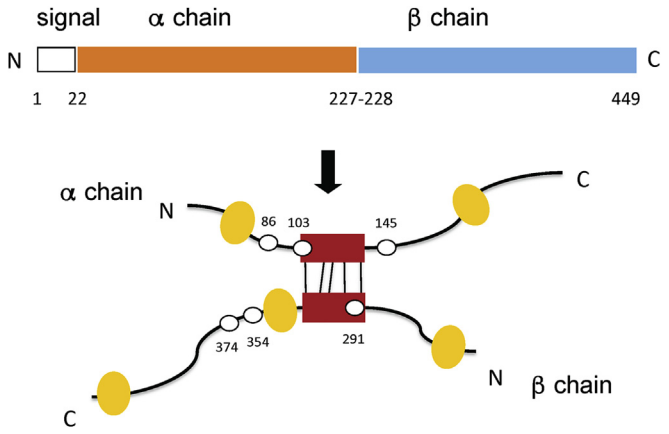

Fig. 1. Predicted human CLU structure. Schematic adapted from (Wilson and Easterbrook-Smith, 2000; Jones and Jomary, 2002; Bailey et al., 2001). The 22-mer secretory signal peptide is proteolytically cleaved from the 449 amino acid precursor polypeptide chain and subsequently the chain is cleaved again between residues Arg227-Ser228 to generate an α-chain and a β-chain. These are assembled in anti-parallel fashion to generate a heterodimeric molecule in which the cysteine-rich centers (red boxes) are linked by five disulfide bridges (black lines) and flanked by five predicted amphipathic α-helices (yellow ovals). The six sites for N-linked glycosylation are indicated (white spots). Amino acid numbering for the N- and C-termini, the cleavage sites, and the sites for N-linked glycosylation are indicated, as in (Kapron et al., 1997).

five amphipathic α-helices (Bailey et al., 2001). The result is a four armed molecule with regions of native disorder, resulting in a dynamic, molten globule-like structure with the capacity to bind a variety of different molecules (Bailey et al., 2001). This includes hydrophobic regions exposed on denatured proteins, important for CLU function as a chaperone (Bailey et al., 2001; Dabbs et al., 2013). CLU also binds a number of specific proteins, including the SC5b-9 complex of complement and immunoglobulins (Wilson and Easterbrook-Smith, 2000). There have been no crystal structure determinations for CLU, and only limited analyses by mass spectrometry (Kapron et al., 1997; Stewart et al., 2007) and nuclear magnetic resonance (Poon et al., 2002a).

3. Biochemical activities and roles in health and disease

3.1. Complement inhibition

Characterized as SP-40,40, CLU was identified in glomerular immune deposits as part of the membrane attack complex of complement (Murphy et al., 1988). Purified CLU was shown to inhibit C5b-6-initiated hemolysis in a dose-dependent manner (Murphy et al., 1989) by binding to complement component SC5b-9 (Choi et al., 1990a). The idea that CLU is a physiological inhibitor of complement-mediated cytolysis was tested using erythrocytes and cells stably transfected with a membrane-anchored form of CLU as targets for complement-mediated cytolysis (Hochgrebe et al., 1999). CLU gave dose-dependent protection of antibody-coated sheep erythrocytes against complement-mediated lysis by diluted normal human serum, however extrapolation to undiluted serum showed that a CLU concentration at least two orders of magnitude greater than its physiological concentration would be needed to confer protection in the circulation (Hochgrebe et al., 1999). Once deposited in tissues however, the effective concentration of CLU may be much higher. The physiologic significance of complement inhibition by CLU remains to be established.