

Clusterin: It's Implication in Health and Diseases

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ABSTRACT

Clusterin (CLU) is a glycoprotein with a nearly ubiquitous tissue distribution that has been reported to be implicated in several physiological processes as well as in many pathological conditions including ageing, diabetes, atherosclerosis, degenerative diseases and tumorigenesis including tumors of prostate, colon, and breast. Two distinct CLU mRNA isoforms, the conventional secreted form of CLU (sCLU) is thought to be a component of high density lipoprotein-cholesterol. sCLU functions as a chaperone for misfolded proteins and it is thought to promote survival by reducing oxidative stress. Nuclear CLU, formed by alternative splicing, is responsible for promoting apoptosis via a Bax-dependent pathway. This review will summarizes our understanding of the importance of CLU in various physiological functions and speculate on its role in disease.

Keywords: Clusterin, Cell Interactions, Complement, Injury, Lipoprotein

Introduction

Apolipoprotein-J (ApoJ), also known as Clusterin (CLU), is 80 k Daglycoprotein with a nearly ubiquitous tissue distribution. It has been implicated in such diverse processes as sperm maturation, regulation of complement activation, programmed cell death, tissue remodeling and lipid transport. Expression of ApoJ is upregulated in acute myocardial infarction, atherosclerosis, myocarditis, oxidative stress, inflammation and after injury in general. [1]Blaschuk et al. in 1983 identified a high-molecular weight protein in ram rete testis fluid and named CLU for its ability to cluster sertoli cells [2]. Additional analyses indicated that this unknown protein was capable of eliciting the "Clustering" of Sertoli cells, mouse testis TM-4 cells, and erythrocytes resulting in the name CLU. In 1984, Griswold and colleagues purified a dimeric acidic glycoprotein from the Sertoli cells of rat testes. [3] In humans, it was firstly purified from serum and the cloned gene was named CLI (complement cytolysis inhibitor) [4] or ApoJ [5] due to similarities with other known apolipoproteins.CLU consists of two polypeptide chains connected by four to five disulfide bonds [6]. The protein is one of the most prominent extracellular chaperones. The chaperone activity of CLU has been intensively studied [7, 8]. With regard to its chaperone activity, CLU is designated as a protein that allows clearing of cellular debris and misfolded proteins, as well as the clearance of $A\beta$ via the blood-brain barrier [9]. The resolute action of chaperone activity, scavenging- and clearance-function, may be the basis for the tissue defensive role of this protein [10]

The Human CLU gene

The *CLU* gene is a single 9-exon expressed at dissimilar levels in virtually all tissues in mammals. *CLU* gene is

located on chromosome 8p21 proximal to the lipoprotein lipase gene locus [11].CLU gene is organized into nine exons, ranging in size from 47 bp (exon I) to 412 bp (exon V) spanning a region of 16580 bp [12]. In humans, at least two distinct CLU mRNA isoforms that differ in their unique exon I exist as a result of alternative transcriptional initiation start sites, (Isoform 1, NM 001831.2 and Isoform 2, NM 203339.1). These two transcripts are probably originated from two transcriptional initiation start sites and only produced in humans and chimpanzees. Both isoforms contain 9 exons (and 8 introns) and a terminal 5'-untranslated region [13]. The mRNA isoform 1 is a major form of CLU mRNA, whereas other forms comprising mRNA isoform 2 account for <1% of total CLU mRNA [14]. This protein is then directed to the lumen of the endoplasmic reticulum by a leader signaling sequence and further undergoes an extensive N-linked glycosylation process, where it becomes a 75-80kDa mature precursor after transportation from the endoplasmic reticulum (ER) to the Golgi apparatus [15].Six N-linked Glycosylation sites of human CLU were identified by Kapron, three on the alpha chain (α 64N, α 81N, α 123N) and three on the beta chain (β 64N, β 127N, β 147N) [16]. The mature 75-80kDa protein undergoes intracellular cleavage between amino acid residue Arg205 - Ser206 to form alpha (α) and beta (β) subunits, which are linked together by five disulfide bonds upon extracellular secretion (sCLU) [17]. Under reduced conditions, the subunits of CLU can be detected with the approximate size of 35-40kDa [18]. But, when cells are subjected to stress, post-translational modifications, such as glycosylation and internal proteolytic cleavage could be blocked [15]. Failure of protein cleavage leads to the

retrograde transport of uncleaved and non-glycosylated (55-60kDa) form of CLU from the Golgi back to the ER. Thus, the secretion of CLU is reduced but not eliminated and intracellular nonglycosylated precursor CLU (pCLU) increased. Another form of CLU is found in the nucleus and is known to be induced after stress, such as ionizing radiation (IR) [15,19]. Many theories for the synthesis of nuclear CLU (nCLU) have been proposed. One theory postulates that CLU is generated from an alternative splicing event, which results in an N-terminal truncated mRNA lacking exon 2, where the ER reader peptide sequence and the first AUG codon reside [19]. This alternative 47 transcript is translated into a precursor protein (pnCLU), which is not cleaved into α or β subunits. Upon cell damage, pnCLU undergoes post-translational modification to form a mature ~55-kDa protein, exposing both of its nuclear localization signals (NLSs), and migrates to the nucleus to induce apoptosis [19].

The functions of CLU

The biological roles of CLU has proved challenging due to two main properties. Firstly its ability to interact with a variety of molecules, including itself, amyloid proteins, lipids, immunoglobulins and components of the membrane attack complex. Secondly, its upregulation during many biological and pathological states. These include developmental remodeling, epithelial cell differentiation, in response to injury and other stresses, as well as in apoptotic disease states.

Clusterin: chaperone activity

CLU, a ubiquitous and highly conserved secreted protein, is an efficient extracellular chaperones (20) CLU potentially inhibits stress-induced protein aggregation by ATP-independent binding to non-native proteins to form soluble, high molecular weight complexes. CLU binds to hydrophobic ligands of misfolded proteins, it was suggested that its interaction with these ligands is as a result of its chaperone properties (21, 22). There is a highly conserved 14-bp element, the heat shock element (HSE), in the CLU proximal promoter in vertebrates which is capable of binding the transcription factor heat shock factor 1 (HSF1) and that then induces CLU expression following heat shock in transient expression assays in cell culture. The conserved HSE motif alone was shown to be capable of driving reporter gene expression in response to heat shock (23).

Clusterin – functions in complement system

CLU protein was initially identified in glomerular immune complexes in a pattern similar to other terminal complement components [24]. Several other reports subsequently showed CLU as a part of the membrane attack complex and has a complement inhibitory role (25,26). Membrane attack complex causes cell lysis by transmembrane channel formation. It is formed on the surface of pathogens as a result of activation of complement system cascade (C1-C9). Based on CLU association with the MAC in some experiments and human diseases, and its ability to inhibit complement mediated cytolysis *in vitro*, an important role for CLU as a complement regulator.

Clusterin function in lipid transport

As an apolipoprotein, CLU is found in a subset of dense HDL particles containing apoA-I and paraoxonase[27]In plasma, CLU forms HDL particles with apoA-I and apoE and may play an important role in reverse cholesterol transport from peripheral tissues to the liver,[28] CLU can promote cholesterol and phospholipid export from macrophage-foam cells which constitute the hallmark cell type of atherosclerotic lesions.[29]The protective effect of sCLU and its peptides is possibly due to the property of CLU in removing toxic and harmful substances to prevent an immune reaction and the subsequent atherosclerotic changes of the vessel wall (30).

Clusterin - cell interactions

CLU is capable of inducing cell aggregation of a variety of cell types hence the name. CLU is considered an adhesion molecule that promotes cell-cell and cell-substratum interactions. CLU may promote these contacts during development when critical cell interactions are taking place, and also during tissue damage hence maintains tissue integrity [31]

Clusterin in neurodegenerative disorders

Genome-wide association studies provide evidence of genetic variation in the CLU gene, CLU in susceptibility of Alzheimer's disease (AD). Its strongest association was found for the common single nucleotide polymorphism (SNP) rs11136000 located in a non-coding, intronic CLU region[32]. CLU expression is highest in the brain and is markedly up regulated under situations of stress and inflammation .This gives rise to glycosylated heterodimeric protein that is constitutively secreted and referred to as soluble clusterin (sCLU), when found in association with lipoproteins which by far we already know are shorter forms of the precursor CLU have been detected intracellularly and named cytosolic, truncated or nuclear CLU [33-35]. The function of intracellular CLU (iCLU) is not completely understood. Of relevance to AD, it has been recently shown that iCLU levels increase quickly in cultured primary neurons exposed to amyloid-b peptides (Ab), and that this iCLU elevation is required for the neurotoxic downstream signaling effects of Ab[36].

sCLU influences Alzheimer's disease (AD) pathogenesis; its levels are found to be raised in AD-affected brain regions[37]. As shown by Western blot and ELISA increased levels of CLUCLare seen in hippocampus, frontal cortex, in cerebrospinal fluid and plasma of AD patients. While there are other studies that demonstrate levels of CLU did not differ significantly between control and AD cases in the frontal cortex, temporal cortex, or thalamus in postmortem human brain[38,39]. On other hand certain other studies are suggestive of elevated plasma CLU levels and association with disease prevalence and severity of AD and with increased amyloid deposition and brain atrophy. Also experimental findings suggest that CLU increases both in amyloid- β (A β) aggregation and clearance, raising the question of whether elevated CLU levels are beneficial or harmful[40]. The explanation for these differences is unclear; the challenge of understanding the role of CLU in AD goes beyond quantification of protein levels partially that can be explained by alternative splicing variants, single nucleotide polymorphisms, and post-translational modifications[41], which are difficult to ascertain with immunological methods and likely associated with development of AD is needed to facilitate clarification of the role of CLU in AD.

Role of clusterin in cancers

CLU, a multifunctional protein have been found to be involved in a number of biological processes like complement cascade, lipid transport, membrane recycling, cell adhesion and programmed cell death[42]. In humans, two proteins: secretory CLU protein (sCLU, secretory CLU) (75-80 kDa) and nuclear CLU protein (nCLU, nuclear clusterin) (55 kDa) encoded by gene isoform 1 ORF [isoform 1, NM 001831, mRNA encodes the functional protein and isoforms 2, NR 038335, non-coding RNA; and isoform 3, NR 045494, non-coding RNA[43]are found on chromosome 8p21-p12[44,45]. It has been found that sCLU enhances tumorigenesis by facilitating binding between Klu70 and apoptotic protein Bax consequently, preventing BAX from localizing to the outer mitochondrial membrane and to stimulate cell death because of its proapoptotic activity[46]. Another mechanism involved in sCLU prosurvival activity is the upregulation of the phosphatidylinositol 3- kinase (PI3K)/protein kinase B (AKT) pathway and insulin-like growth factor (IGF)-1 activating PI3K/AKT pathway through upregulation of sCLU[47], sCLU has other actions related to tumor behavior. For example, in clear cell renal carcinoma, sCLU regulates tumor cell migration, invasion and metastasis by modulating extracellular signal-regulated kinase (ERK)1/2 signaling and matrix metallopeptidase (MMP)-9 expression[48]. In epithelial ovarian cancer, CLU has been observed to promote angiogenesis and chemo resistance. Other pathways CLU participates in to downplay apoptosis in tumor cells include the PI3K/AKT/mTOR pathway and NF-κB pathway.As evident by its varied key roles in cancer development, CLU can serve as a therapeutic target for fighting tumor growth and chemoresistance[49].

Conclusion

CLU has been implicated as a significant risk factor for the development of numerous diseases, however, an extensive gap exists in the literature in understanding functions of CLU. As CLU isoforms appear to mediate various physiological processes, the tendency to focus on the effects of CLU as a singular protein could lead to conflicting reports in the literature that are currently unresolved. Therefore, before researchers can fully ascertain the therapeutic potential of CLU from a clinical perspective, it is imperative that key deficiencies are addressed at the genetic level.

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