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MOLECULAR ASPECTS OF APOPROTEIN J

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ABSTRACT

Apolipoprotein J (apoprotein J, apo J, clusterin) is a multifunctional protein normally associated with lipids in plasma and cerebrospinal fluid, and secreted as lipoparticles by hepatocytes and astrocytes. Apo J is abundant in numerous biological fluids including semen, urine, breast milk, plasma and cerebrospinal fluid. There are two major sources of apo J in the circulation: plasma, in which it is found associated with high-density lipoprotein (HDL) particles; and platelets, where it is a constituent of the α-granules. Apo J has been implicated in a wide range of physiological and pathophysiological processes, such as reverse lipid transport and redistribution, apoptosis, folding of damaged extracellular proteins (chaperone), cell adhesion and aggregation, membrane recycling, complement regulation, tissue remodeling, tumorigenesis and several age related diseases (e.g. atherosclerosis and Alzheimer's disease). In blood, complexes of apo J with apolipoprotein A-I and the human esterase paraoxonase regulate the transport and local redistribution of lipids. Serum apo J concentrations are increased in experimental models of diet-induced atherosclerosis, as well as in patients with diabetes mellitus or coronary heart disease. However, the pathophysiological role of apo J in atherosclerosis and the therapeutic implications merit further evaluation.

KEYWORDS: Apolipoprotein J, clusterin, atherosclerosis, HDL, cardiovascular disease, plasma.

APOLIPOPROTEINS

Apolipoproteins or apoproteins are the polypeptides found in various types of lipoproteins. Earlier, the existence of three major groups of apolipoproteins – Apo A, Apo B and Apo C

were reported[1], but lately, more apolipoproteins, such as D, E, H and J have been characterized. The classification of apolipoproteins based on their various characteristics, physiological function and chromosomal locations is summarized in Table 1. The gene of apolipoproteins are located on chromosomes 1, 2, 3, 6, 11 and 19 (Table 1). All these apolipoproteins are associated with lipoproteins and involved in the transport of chylomicrons, triglyceride, cholesterol, fatty acids, etc. They also act as cofactors or activators of enzymes like lecithin-cholesterol acyl transferase (LCAT) and lipoprotein lipase (LPL). They are differently implicated in various diseases and play significant role in diagnosis and prognosis of several disease conditions.

Table 1:- Classification and properties of major human plasma apolipoproteins

Apolipoprotein	Chromosomal location	Functional activity	Lipoprotein carrier(s)
Apo A-I[2]	11	Cofactor LCAT	Chylomicron, HDL
Apo A-II[2]	1	Not known	HDL
Apo A-IV[3]	11 2	Activation of LCAT, Secretion of triglyceride from liver binding protein to LDL receptor	Chylomicron, HDL VLDL, IDL, LDL
Apo B-48[4]	2	Secretion of triglyceride from intestine	Chylomicron
Apo B-100[4]	2	Cholesterol transport from liver	LDL
Apo C-I[4]	19	Activation of LCAT (?)	Chylomicron, VLDL, HDL
Apo C-II[5]	19	Cofactor of LPL	Chylomicron, VLDL, HDL
Apo C-III[6]	11	Inhibition of apo CII, activation of LPL	Chylomicron, VLDL, HDL
Apo D[2]	3	Unknown	HDL
Apo E[7]	19	Facilitation of uptake of chylomicrons remnant and LDL	Chylomicron, VLDL, HDL
Apo (a)[8]	6	Unknown	Lp (a)

HISTORY AND NOMENCLATURE

Petar Alaupovic, an internationally renowned biochemist and lipidologist, was a founder of the modern field of lipoproteins whose ideas continue to have an impact on its development. He proposed the ABC nomenclature for apolipoproteins, which initially included apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D, and E. Dr Alaupovic showed that these lipoprotein subtypes have specific functions and relation to atherosclerosis.[9]

Originally, clusterin was identified as a major constituent of ram rete testis fluid and shown to induce the aggregation of Sertoli cells *in vitro* (hence named “clusterin”) and favor homotypic aggregation within mixed cell suspensions.[10,11] Subsequent researcher shows that

clusterin is similar to apoJ. Besides apoJ and clusterin, other synonyms include complement lysis inhibitor (CLI), Ku70-binding protein 1 (KUB-1), sulfated glycoprotein 2 (SGP-2), Testosterone-repressed prostate (TRPM-2).[12]

GENETICS

CLU in humans is encoded by a single copy-gene, which is located on chromosome 8p21. The wide distribution in different tissues indicates the importance of its biological roles.[12] CLU is involved in lipid transport since it is associated with high-density lipoproteins (HDL) in plasma, specifically with particles containing apolipoprotein A-1 and cholesteryl ester transfer protein (CETP).[13] It has been shown that induces cholesterol export from macrophage foam cells,[14] transports lipids during cell differentiation and cell death[15] and stabilizes stressed proteins.[16]

STRUCTURE AND LOCATION

It is a 70 kDa glycoprotein, circulating as disulphide linked heterodimer component of lipid poor HDL and VLDL. Its function is not clear, but it is thought to be involved in lipid transport, regulation of complement function, sperm maturation and membrane recycling.[14] The structure of apoJ/clusterin has not provided much insight into function. Mammalian apoJ/clusterins are approximately 80-kDa heterodimers[17,18] consisting of two 40-kDa chains joined by a unique five-disulfide-bond motif.[19] The protein has limited homology to other proteins and lacks clear functional motifs.[17] It does contain three putative amphipathic α-helical regions, which could allow it to interact with lipids and hydrophobic regions of other proteins.[20] A number of studies presented that is implicated in aging and age-related diseases as neurodegeneration, diabetes and atherosclerosis[21-23] and acts as a biomarker of cellular senescence and oxidative stress.[24]

Human apoJ has three such domains, one in Jα and two in Jβ.[25] These domains are not, however, homologous with the 22-mer repeat that constitutes the amphipathic helices of members of the apolipoprotein gene family.[26] Unlike other apolipoproteins but similar to certain coagulation and complement proteins, apoJ circulates as a disulfide-linked heterodimer for which the subunits, Jα and Jβ, are produced by proteolytic cleavage of the apoJ precursor.[25,27,28] Each apoJ subunit is glycosylated via Asn, and carbohydrate accounts for 30% of the molecular mass. Moreover, apoJ shares some homology with complement components C7, C8, and C9, specifically within a Cys rich motif in Jar (residues 75-98).[29]

Plasma apoJ-lipoproteins are spherical particles[14,30] that have a bimodal distribution within HDL_2 and HDL_3+VHDL classes in the density range 1.16-1.25 g/ml.[14] The apoJ-containing species are relatively poor in lipid: protein makes up 78-89%, and lipid 11-22% of the lipoprotein mass.[14,30] Of the lipids, phospholipid and cholesterol predominate; triglyceride accounts for about 1% of apoJ-HDL lipid.[14,30] The major proteins are apoJ and apoA-I; the mole ratio of apoJ: apoA-I present in affinity-purified apoJ-HDL is 5:l.[31] The apoA-I associated with apoJ represents only 2-4% of the total apoA-I in plasma[14,30] and is tightly associated with apoJ, requiring nonionic detergents for dissociation.[30] The origin of blood apoJ-HDL, present at ~10 mg/dl,[29,32] is not known. The relatively high abundance of apoJ mRNA in hepatocytes[25] combined with the large size of the liver predict that the liver, rather than other organs that also express apoJ mRNA,[25] is the source of the circulating pool of apoJ.

In rodents, apoJ becomes bound to the heads and distal tails of spermatozoa, suggesting its participation in sperm maturation.[33,34] ApoJ can also bind to membranes of other cells, notably adrenal chromaffin cells[35] and erythrocytes,[36] where it has been implicated in the processes of membrane retrieval[37] and cell-cell association, respectively. In addition, apoJ is up regulated in tissues undergoing programmed cell death and degeneration.[38] ApoJ-lipoproteins secreted into the blood may circulate without further modification. Alternatively, they may be secreted as species quite different in macromolecular structure and composition from those in plasma, and be converted to the plasma species by plasma enzymes and/or lipid transfer proteins. The differences in overall size and in lipid: protein ratios between Hepatocellular carcinoma (Hep G2) cell and plasma apoJ-lipoproteins suggest that HepG2 cells secrete "nascent" apoJ-lipoproteins that lose lipid, particularly triglyceride, and accumulate apoA-I as they circulate. Alternatively, circulating lipoproteins comprised of both apoJ and apoA-I may be derived from tissues other than the liver. Consistent with the concept of apoJ-lipoprotein remodeling is the finding that purified apoJ, added to apoJ-deficient plasma, binds apoA-I.[31]

The associated apoA-I has the potential to alter the half-life of apoJ-lipoproteins in the circulation by stabilizing them or by blocking their uptake and clearance, with a consequent increase in apoJ levels. Although the lipid compositions of plasma apoJ-HDL and nascent HepG2 apoJ-lipoproteins are similar in that both contain significant phospholipid and cholesterol, HepG2 cell apoJ-lipoproteins contain significantly more triglyceride. HepG2 cell apoJ-lipoproteins secreted over a period of 6 h have a phospho1ipid: triglyceride ratio (2:1),

similar to that of apoE-lipoproteins (3: l) but significantly greater than that of apoA-I-containing lipoproteins (7-8: l). Taken together, these findings suggest that apoJ is secreted by the liver as a triglyceride-rich lipoprotein that is transformed during circulation to a triglyceride-poor species. The corollary of this suggestion is the prediction that the triglyceride in nascent apoJ-lipoproteins is a substrate for LPL, HTGL, and/or cholesteryl ester transfer protein (CETP).

Clusterin expression is increased during pathological stresses (*e.g.* hydrostatic pressure insult or ischemic injury in the kidney) and certain disease states (*e.g.* gliomas).[12] A variety of independent studies have suggested that clusterin protects cells from stresses such as tumor necrosis factor, heat, and oxidative stress.[39] Furthermore, recent studies of clusterin knock-out mice have suggested that clusterin protects mice from (i) the pathological consequences of inflammation associated with experimentally induced autoimmune myocarditis[40] and ischemia,[41] although the latter claim has been disputed,[42] and (ii) age-dependent deposition of antibody-containing aggregates in the kidney.[43] The clusterin promoter contains a highly conserved 14-bp element, which is recognized by the transcriptional regulator heat shock factor 1.[44] Heat shock factor 1 activates expression of heat shock proteins (which protect cells from stresses) and clusterin.[44] An emerging theme is that clusterin is a protective molecule that is up-regulated during times of physiological stress.

A comparison of the sequences of clusterin from eight different mammals shows that there are five highly conserved histidine residues within residues 241 and 290 of the protein (human clusterin numbering). Thus, His-252 and His-263 are found in all of the available sequences, whereas His-290, His-241, and His-261 are also highly conserved, being found in a minimum of five of the eight sequences. It is therefore possible that this region of clusterin represents an “electrostatic switch”; pH-dependent protonation of its histidine residues may lead to disruption of the interfaces between heterodimers within clusterin aggregates, favoring dissociation of the aggregates.

FUNCTIONS

Apolipoprotein J (apoJ)/clusterin is a circulating glycoprotein constitutively expressed by diverse epithelial cells. The protein is induced in injured organs in various disease states, such as Alzheimer’s disease, atherosclerosis, myocardial infarction, and multiple forms of acute and chronic renal disease.[45,46] Proposed functions for apoJ/clusterin include lipid transport, complement defense, regulation of apoptosis, membrane protection, and promotion of cell-

cell interactions.[46] ApoJ/clusterin can bind a large number of macromolecules implicated in disease initiation and progression, including immunoglobulins and complement components. Recently clusterin has been demonstrated to function as a molecular chaperone, preventing denatured protein precipitation through binding to exposed hydrophobic regions and improving high-molecular weight complex solubility.[47]

In addition to its association with lipoproteins, apoJ has been isolated from human plasma in association with soluble complexes of the terminal complement cascade components, C5b-9.[20] Its association with C5b-9 complexes implies a role in complement function and, in fact, apoJ is a potent inhibitor of complement-mediated cell lysis in vitro by interacting with C5b-7 to prevent activation of C8 and C9.[48,49] Potential roles in both lipid metabolism and complement function are substantiated by structural considerations.

ApoJ and its homologs are thought to participate in biological functions other than lipid transport and complement regulation (Table 2). ApoJ is particularly abundant in the male reproductive tract where it is secreted by testicular Sertoli cells and epididymal epithelium.[33]

Table 2: Apo J homologs

Name	Species	Comments
Apolipoprotein J	human	HDL associated protein
NA1/NA2	human	HDL associated protein
SP-40, 40, CLI	human	Complement cell lysis inhibitor
TRPM-2	rat	Programmed cell death
SGP-2, DAG	rat	Reproductive tract; Sertoli cell secretory protein
S45-S35	rat	Sperm binding protein
Clusterin	ram	Cellular aggregation
gp80	dog	MDCK apical secretory protein
Glycoprotein III	cow	Adrenal medullary chromaffin granule secretory protein

The exact role of apoJ-lipoproteins in whole body lipid metabolism and homeostasis is unknown at present. The possibility that apoJ may bind apoA-I after entering the circulation leads to speculation about the circumstances of an interaction between them. The apoA-I that binds to apoJ-lipoproteins may be lipid-deficient apoA-I derived from several sources: secreted from hepatocytes or shed from triglyceride-rich lipoproteins as a consequence of lipoprotein lipase (LPL) or hepatic triglyceride lipase (HTGL)-mediated triglyceride metabolism.[50,51] This latter potential source is intriguing in light of the positive correlation between plasma triglyceride and apoJ levels.[32] Since high levels of triglyceride-rich lipoproteins and increased lipolysis can result in a rise in the level of lipid deficient apoA-I,

apoJ may serve as a ‘‘sink” for apoA-I shed from these lipoproteins. *In vivo*, the distribution of clusterin within tissues is broad; clusterin mRNA being relatively abundant in testes, brain, liver, and ovary and detectable in several other tissues including the kidney, thymus, spleen, and heart. Various functional aspects of apoJ/clusterin are discussed below.

1. Cardiovascular System

The role of CLU in atherosclerosis remains largely unknown. Since cellular stress is engaged with the pathogenesis of the disease, the study of the apolipoprotein expression on vascular tissue and the elucidation of underlying mechanism merit scientific interest. Studies have shown that CLU distribution in human aorta is increased with the progression of atherosclerosis indicating a protective response to oxidative stress.[13,52,53] Additionally to its roles as secreted protein, it has been reported that might act intra-cellularly regulating homeostasis in human cells.[54] High serum concentration of CLU has been coupled to vascular damage and generalized stress conditions such as type II diabetes and coronary artery disease[23] and it has been associated with significant coronary stenosis.[55]

In aortic tissue, CLU expression increases with atherosclerosis progression from fatty streaks to advanced lesions[13,52] while its presence in normal aortas is unobtrusive.[13] The molecule localization on vascular tissue could be attributed to HDL particles penetration from plasma, production through vascular smooth muscle cells (VSMC), release from activated platelets and retention in vascular wall through glycoproteins of extracellular matrix.[56] Recently, it has been shown that CLU expression in VSMC is induced by cellular RNA released from necrotic cells in atherosclerotic lesions through toll-like receptor 3[53] while it seems that inhibits the apoptosis of VSMC in vitro by binding to modified-LDL particles[57] The enhanced expression of CLU on injured tissue may serve the clearance of necrotic cell debris contributing to its protective role.

CLU expression was not higher in diabetic patients or in patients suffering from coronary artery disease. Studies have demonstrated that serum CLU concentration is elevated in these patients’ categories[23,55] reflecting a generalized stress induction mechanism, which is related to these diseases. Certainly, it was supported that the elevated serum levels indicate vascular damage.

2. Immunity

A role for apoJ/clusterin in immune complex-mediated disease was first suggested by its interaction with immunoglobulins. apoJ/clusterin can bind to the Fc and Fab regions of all isotypes of IgG, IgM and IgA by a noncovalent mechanism.[58] The site of interaction is different than the Fc binding site of C1q and protein A.[58] apoJ/clusterin has been shown to preferentially bind to aggregated compared to monomeric IgG.[58] These results suggest that apoJ/clusterin has multiple potential binding sites through which it may interact and facilitate the clearance of polymeric IgG present in immune complexes.

In addition to its interaction with immunoglobulins, apoJ/clusterin has been found in conjunction with immune deposits in a number of immune-mediated glomerular diseases, including IgA nephropathy, membranous glomerulonephritis, and lupus nephritis.[59,60] In most cases apoJ/clusterin colocalizes with components of the membrane attack complex when these components are present with immunoglobulins but not when the membrane attack complex is found in the absence of immunoglobulins, suggesting a direct role for apoJ/clusterin in the processing of immune complexes.[59,60] apoJ/clusterin has also been localized to the glomerulus in such immune-mediated models of glomerulonephritis as Heymann nephritis and anti-Thy 1 nephritis.[61,62] In the latter model, upregulation of both clusterin mRNA and protein has been demonstrated in mesangial cells, providing evidence that clusterin can be synthesized by these cells following immune attack.[62] The association of apoJ/clusterin with immune deposits in experimental and human glomerulonephritis suggests it may modulate responses to immune-complex-induced injury. An association between apoJ/clusterin and immune complex disease is found in patients with systemic lupus erythematosis (SLE). Levels of apoJ/clusterin in serum are lower in patients with SLE than in normal controls or patients with rheumatoid arthritis, osteoarthritis, or Sjogren's syndrome.[63] ApoJ and known apolipoproteins have in common the predicted amphipathic helices that are important in protein-lipid interactions.[25,27]

Some of clusterin's known properties seem to be explained by its avidity for exposed hydrophobic domains on macromolecules. First, clusterin, which is a potent inhibitor of complement mediated lysis *in vitro*,[19,64,65] and colocalizes with membrane attack complex (MAC) deposits *in vivo*,[20,60,59,66] most probably binds to newly exposed hydrophobic domains in nascent C5b-7, C5b-8, and C5b-9 complexes thereby inhibiting membrane insertion.[67] Second, clusterin associates with certain high density lipoprotein (HDL)

particles[14,68,30] and is dissociated from these particles by non-ionic detergents, suggesting that it interacts directly with lipids.[30] There is growing evidence that clusterin may be involved in tissue remodeling. Clusterin gene expression is highly increased during the involution of certain tissues in response to hormonal modulations or injury,[38,69,70,71,72,73] in particular under circumstances where cell death occurs by apoptosis. Recent reports indicate, however, that clusterin is not expressed by apoptotic cells *in vivo*,[21,74] and it has been suggested that clusterin may be part of the repair and remodeling response within regressing tissues.[12] Recently, clusterin gene expression has been correlated to the *in vitro* differentiation of aortic smooth muscle cells[65] and the oncogenic transformation of neuroretinal cells by various retroviral oncogenes.[75]

3. Oxidative stress

The chaperone action of clusterin might be physiologically "protective" by reducing the rate or extent of progression of diseases associated with abnormally high levels of protein precipitation. Many features of the chaperone action of clusterin are similar to that of the intracellular small heat shock proteins (sHSPs).[11] For example, both types of chaperone interact specifically with stressed proteins that are slowly aggregating on the off-folding pathway toward a precipitated state[64] to form stable, solubilized high molecular weight complexes.[39,22] They do not themselves refold stressed proteins but, by binding to them, create a refolding-competent reservoir from which other ATP-dependent chaperones may retrieve functional proteins.[17] The quaternary structure and chaperone action of at least some of the sHSPs is affected by increased temperature. For example, elevated temperature induces an increased rate of subunit exchange in mammalian αA-crystallin and a concomitant enhancement of its chaperone action.[76] Similarly, at low to physiological temperatures, yeast HSP26 exists in solution as oligomers.

Clusterin/Apolipoprotein J is a secreted protein biosensor of oxidative stress, which is unregulated in a wide variety of pathological processes including aging, neurodegeneration, diabetes and atherosclerosis.[23,24] Although the precise function of the molecule is still under investigation, it has been accepted that CLU exerts cytoprotective and anti-inflammatory actions.[53] Clusterin has been implicated in pathological conditions in which oxidative stress plays a central role such as aging, neurodegenerative diseases, and cancer progression.[77] Based on its large repertoire of unrelated binding partners, it has been also implicated in diverse physiological processes such as lipid transport, cell differentiation, regulation of

apoptosis, clearance of cellular debris, and stabilization of misfolded proteins.[77] Both cytoprotective and cytotoxic roles for clusterin have been reported. Functionally, clusterin is similar to the small heat shock proteins with chaperone-like activity, binding to stressed and misfolded proteins.[22] Much of the work on clusterin has focused on its role as an extracellular chaperone,[78,79] binding to exposed hydrophobic regions of proteins and maintaining them in a state competent for subsequent refolding by other chaperones, *e.g.* HSP70.[80,81,16]

The role of clusterin as an extracellular chaperone is well established.[77,79,82] For example, its interaction with β-amyloid promotes β-amyloid clearance and uptake (via cell surface megalin receptor) and subsequent degradation.[82] Recent association of single nucleotide polymorphisms at the *CLU* gene locus with Alzheimer disease[83,84] supports the suggestion that both *CLU* and *APOE* may act as modifying genes to cooperatively regulate the deposition and clearance of β-amyloid,[85] which may affect the onset and/or clinical expression of the disease. Similarly clusterin and COMMD1, by affecting the efficiency of clearance of mutant Cu-ATPase molecules, may play a role in modifying the clinical expression of Menkes and Wilson diseases. Whether they function cooperatively or redundantly remains to be established. Recently, the pharmacological folding chaperones such as 4-phenylbutyrate and curcumin showed potential to rescue the folding defects of ATP7B harboring patient mutations[86] and may overcome some of the effects of variations in these potential modifying genes.

Intracellular (nuclear and cytosolic) forms of clusterin exist, but the mechanisms responsible for the derivation of these forms remain poorly defined, and less is known about its intracellular role.[77,79] Clusterin potentially represents the only chaperone that regulates protein stability both extra and intracellularly.[77] Therefore, the Cu-ATPases provide a new model toward understanding the role of intracellular clusterin. This work also provides an insight into the mechanisms of Cu-ATPase quality control, which is necessary for the maintenance of normal copper homeostasis and cell survival in disease states in the context of continuous synthesis of mutant Cu-ATPase molecules. Overall, the results from this study support the possibility that variations in clusterin alleles could contribute to the variability in the clinical expression of Menkes and Wilson diseases.

The enhanced ligand-binding and chaperone actions of clusterin at low pH may have important physiological relevance. A phenomenon known as local acidosis occurs at sites of tissue damage or inflammation where the local pH falls to <6. This phenomenon has been reported to occur at sites of inflammation,[87] cardiac ischemia,[88] and infarcted brain[89] and in the brains of Alzheimer's sufferers.[90] Under these conditions, clusterin oligomers may dissociate, and the enhanced binding/chaperone actions of the 80-kDa species could help to inhibit the aggregation and deposition of inflammatory and/or toxic insoluble protein deposits, which would otherwise exacerbate pathology.

4. Erythrocytes

sCLU is a primarily secreted protein sorted for secretion through the classic endoplasmic reticulum- and Golgi-associated secretory pathway.[54,91,92,93] There is evidence that sCLU is also a "secreted" component of mature RBCs through the membrane exovesiculation process. sCLU contributes to the scavenging of oxidized or aggregated molecules that are selectively removed from senescent or stressed RBCs via vesiculation. By assuming that, sCLU represents not only a molecular biomarker of cellular senescence and oxidative stress but also a pro-survival factor that contributes to the transient inhibition or delay of the premature removal of otherwise functional RBCs from the circulation; this proposed function of sCLU is further supported by the recently predicted role of the protein in the RBCs death regulatory pathways.[94]

A possible mechanism for the chaperone action of clusterin is schematically represented in Fig. 1. This scheme is analogous to that proposed for the sHSPs[95] and draws on the conclusions of recent work demonstrating that clusterin binds preferentially to slowly aggregating proteins on the off-folding pathway.[64] The unfolding of a target protein under stress conditions occurs via a series of partly structured intermediates or molten globule states that are present along the normal protein folding pathway. These intermediates are relatively long-lived and potentially unstable, because they expose significant hydrophobicity to solution, which may facilitate their aggregation and precipitation via the irreversible off-folding pathway. A protein that is present on the off-folding pathway undergoes dynamic processes of association and dissociation. In solution, clusterin also exhibits reversible association to form aggregates of various sizes.[96]

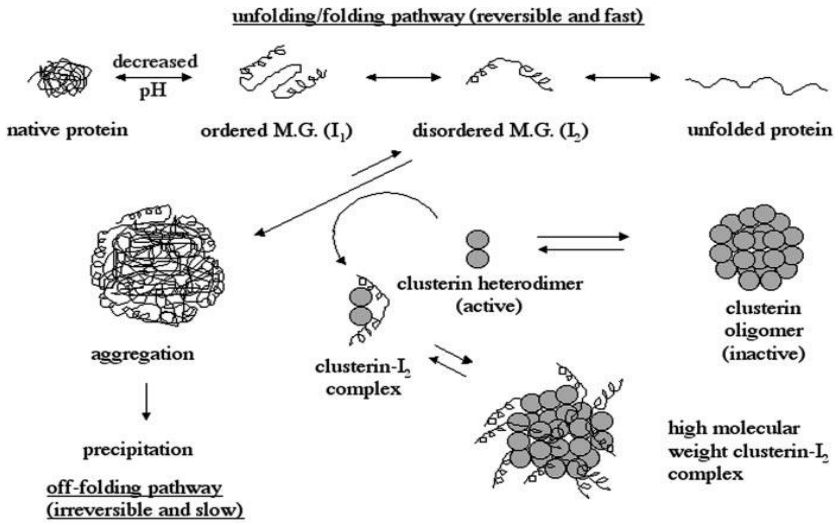

FIG. 1: A schematic representation of a possible mechanism for the chaperone action of clusterin.

5. Nervous system

In the nervous system, a close relationship between neurodegeneration and clusterin gene expression has been established.[97,98] Although several normal populations of neuronal and glial cells contain clusterin mRNA,[97,99] higher clusterin mRNA levels are observed after deleterious experimental treatments, like surgical,[100,101] or excitotoxic[97,99] brain injuries, but also in pathological brains from Alzheimer's diseased human patients,[73] scrapie infected hamsters,[102] or at epileptic foci.[103] Clusterin mRNA accumulation has also been observed in retinitis-pigmentosa,[104] and shown to coincide with the time of photoreceptor cell deaths in mouse models of this disease.[105] Purkinje cells whose apoptosis is induced by the lurcher gene, were shown to contain high levels of clusterin mRNAs prior to their death .[106] Besides, the clusterin protein has been found associated with dystrophic neurites,[107] amyloid plaques[108] and the soluble form of beta amyloid protein[109] from Alzheimer's diseased human brains. The ischaemic, but not the normal, human Purkinje cells are also intensely immunostained with anti-clusterin antibodies.[110] In spite of these numerous observations, the precise function of clusterin in damaged nervous system remains to be elucidated.

6. Genito-urinary system

Clusterin is induced during renal and other tissue injuries.[12,97,111-113] Despite its immediate and often prominent recruitment after injury, the role of clusterin remains elusive. Persistent increased expression of clusterin occurred in several chronic models of renal disease including renal ablation, tubulointerstitial disease induced by dietary deficiency of vitamin E and selenium, and in a mouse model of polycystic kidney disease.[114-116] Studies in human

renal disease have demonstrated clusterin predominantly in glomerular immune deposits usually in association with other complement components.[60,66] Tubular staining for clusterin has either not been a prominent feature of the diseases studied[60] or details regarding such tubular staining have not been provided.[66] Neither of these studies examined clusterin in cystic disorders. Despite the marked tubular epithelial cell induction of clusterin in experimental models of renal injury, limited details regarding tubular expression of clusterin in human renal disease are available.

The earliest studies of clusterin were done in the reproductive system where clusterin was identified as a major protein in the secretory products of cultured rat Sertoli cells and the fluid of ram rete testis.[117,10] In the rat testis, clusterin is synthesized by Sertoli cells, secreted into the lumen of the seminiferous tubules, and is the dominant protein in the spent medium of cultured rat Sertoli cells.[118] Clusterin is also localized to the prostate. Tenniswood and co-workers found that after castration of rats, expression of clusterin or testosterone-repressed prostate message-2 (TRPM-2) increased in the ventral prostate[119] in relation to cellular damage. Clusterin has been localized to the cytoplasm of epithelial cells in the proximal region of the prostatic duct.[71] Also, the prostate form of clusterin is glycosylated differently than testicular and epididymal clusterin.[120] Although prominent in the entire reproductive tract of the male, less is known about where clusterin is expressed in the female reproductive tract. Northern analysis showed that clusterin mRNA is expressed in the human ovary and the surface epithelia of the uterus and the uterine glands.[121]

CONCLUSIONS AND FUTURE PERSPECTIVES

Apolipoprotein J (apoJ)/clusterin is a circulating glycoprotein constitutively expressed by diverse epithelial cells. Apo J has been implicated in a wide range of physiological and pathophysiological processes, such as reverse lipid transport and redistribution, apoptosis, folding of damaged extracellular proteins (chaperone), cell adhesion and aggregation, membrane recycling, complement regulation, tissue remodeling, tumorigenesis and several age related diseases (e.g. atherosclerosis and Alzheimer's disease). Further study is required to fully understand the complex pathophysiological role of apoJ/clusterin.

REFERENCES

1. Gustafson A, Alaupovic P, Furman RH. Studies of the composition and structure of serum lipoproteins. Separation and characterization of phospholipid-protein residues obtained by partial delipidization of very low density lipoproteins of human serum. Biochemistry, 1966; 5: 632-40.
2. Law SW, Lackner KJ, Fojo SS, Hospattankar A, Monge JC, Brewer HB Jr. The molecular biology of human apo A-I, apo A-II, apo C-II, apo B. In Argel A and Frolich J: Lipoprotein deficiency syndromes. Advances in Exp Med and Biology, New York; Plenum Press: 1986, pp. 201.
3. Silvia SF, Jeffery M, Gred A, Bryan H. Lecithin cholesterol acyltransferase deficiency and fish eye disease (8th edn.). The metabolic and molecular bases of inherited disease. London; McGraw-Hill: 2001, 118, pp. 2817-2833.
4. Scott J. RNA editing: a novel mechanism for the regulation of dietary cholesterol absorption. The Humphry Davy Rolleston lecture 1989. J R Coll Physicians Lond, 1990; 24: 101-106.
5. Calvert GD, Abbey M. Plasma lipoproteins and apoproteins and proteins concerned with lipid metabolism. Adv Clin Chem, 1985; 24: 217-98.
6. Montine KS, Bassett CN, Ou JJ, Markesbery WR, Swift LL, Montine TJ. Apolipoprotein E allelic influence on human cerebrospinal fluid apolipoproteins. J Lipid Res, 1998; 39: 2443-2451.
7. Lusis AJ. Genetic factors affecting blood lipoproteins: The candidate gene approach. J Lipid metabolism Adv Clin Chem, 1988; 29: 397-429.
8. Carl A Burtis, Edward R. Tietz's Fundamentals of Clinical Chemistry (5th edn.). Lipid Lipoprotein and Apoprotein, Sydney; WB Saunders: Chap 24, pp. 462-492.
9. Sacks FM, Brewer HB. Petar Alaupovic: The father of lipoprotein classification based on apolipoprotein composition. Arterioscler Thromb Vasc Biol, 2014; 34: 1111-3.
10. Blaschuk O, Burdzy K, Fritz IB. Purification and characterization of a cell-aggregating factor (clusterin), the major glycoprotein in ram rete testis fluid. J Biol Chem, 1983; 258: 7714-7720.
11. Kapron JT, Hilliard GM, Lakins JN, Tenniswood MPR, West KA, Carr SA, et al. Identification and characterization of glycosylation sites in human serum clusterin. Protein Sci, 1997; 6: 2120–2133.
12. Jenne DE, Tschopp J. Clusterin: the intriguing guises of a widely expressed glycoprotein. Trends Biochem Sci, 1992; 17(4): 154–159.

13. de Silva HV, Stuart WD, Duvic CR, Wetterau JR, Ray M, Ferguson DG, et al. A 70 kDa apolipoprotein designated ApoJ is a marker for subclasses of human plasma high density lipoproteins. J Biol Chem, 1990; 265: 13240–13247.
14. Gelissen IC, Hochrebe T, Wilson MR, Easterbrook-Smith SB, Jessup W, Dean RT, et al. Apolipoprotein J (clusterin) induces cholesterol export from macrophage-foam cells: a potential anti-atherogenic function? Biochem J, 1998; 331(1): 231–237.
15. Ahuja HS, Tenniswood M, Lockshin R, Zakeri ZF. Expression of clusterin in cell differentiation and cell death. Biochem Cell Biol, 1994; 72(11–12): 523–530.
16. Poon S, Easterbrook-Smith SB, Rybchyn MS, Carver JA, Wilson MR. Clusterin is an ATP-Independent chaperone with very broad substrate specificity that stabilizes stressed proteins in a foliding-compentent state. Biochemistry, 2000; 39: 15953–15960.
17. Jenne DE, Tschopp J. Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: identity to sulfated glycoprotein 2, a constituent of rat testis fluid. Proc Natl Acad Sci USA, 1989; 86: 7123–7127.
18. Murphy BF, Kirszbaum L, Walker ID, d'Apice AJF. SP-40, 40 a newly identified normal human serum protein found in the SC5b-9 complex of complement and in the immune deposits in glomerulonephritis. J Clin Investig, 1988; 81: 1858–1864.
19. Kirszbaum L, Bozas SE, Walker ID. SP-40, 40, a protein involved in the control of the complement pathway, possesses a unique array of disulphide bridges. FEBS Lett, 1992; 297: 70–76.
20. Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR. Clusterin has chaperone-like activity similar to that of small heat shock proteins. J Biol Chem, 1999; 274: 6875–6881.
21. Garden GA, Bothwell M, Rubel EW. Lack of correspondence between mRNA expression for a putative cell death molecule (SGP-2) and neuronal cell death in the central nervous system. J Neurobiol, 1991; 22(6): 590–604.
22. Mackness B, Hunt R, Durrington PN, Mackness MI. Increased immunolocalization of paraoxonase, clusterin and apolipoprotein A-1 in the human artery wall with the progression of atherosclerosis. Arterioscler Thromb Vasc Biol, 1997; 17(7):1233–1238.
23. Trougakos IP, Poulakou M, Stathatow M, Chalikia A, Melidonis A, Gonos E. Serum levels of the senescence biomarker clusterin/apolipoprotein J increase significantly in diabetes type II and during development of coronary heart diseases or at myocardial infarction. Exper Gerontol, 2002; 37:1175–1187.

24. Antonelou MH, Kriebardis AG, Stamoulis K, Trougakos IP, Papassideri IS. Apolipoprotein J/Clusterin is a novel structural component of human erythrocytes and a biomarker of cellular stress and senescence. Plos One, 2011; 6(10):1–10.
25. de Silva HV, Harmony JAK, Stuart WD, Gil CM, Robbins J. Apolipoprotein J: structure and tissue distribution. Biochemistry, 1990; 29: 5380-5389.
26. Li WH, Tanimura M, Luo CC, Datta S, Chan L. The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships, and evolution. J Lipid Res, 1988; 29: 245-271.
27. Collard MW, Griswold MD. Biosynthesis and molecular cloning of sulfated glycoprotein 2 secreted by rat Sertoli cells. Biochemistry, 1987; 26: 3297-3303.
28. Burkey BF, de Silva HV, Harmony JAK. Intracellular processing of apolipoprotein J precursor to the mature heterodimer. J Lipid Res, 1991; 32: 1039-1048.
29. Kirszbaum L, Sharpe JA, Murphy B, d'Apice AJE, Classon B, Hudson P, et al. Molecular cloning and characterization of the novel, human complement-associated protein, SP-40, 40: a link between the complement and reproductive systems. EMBO J, 1989; 8: 711-718.
30. Jenne DE, Lowin B, Peitsch MC, Bottcher A, Schmitz G, Tschopp J. Clusterin (complement lysis inhibitor) forms a high density lipoprotein complex with apolipoprotein A-I in human plasma. J Biol Chem, 1991; 266:11030-11036.
31. Stuart WD, Krol B, Jenkins SH, Harmony JAK. Structure and stability of apolipoprotein J-containing high density lipoproteins. Biochemistry, 1992; 31: 8552-9.
32. Jenkins SH, Stuart WD, Harmony JAK, Kaplan LA. Development of competitive enzyme-linked immunosorbent assay (ELISA) for a new apoprotein (J). Clin Chem, 1990; 36: 963.
33. Sylvester SR, Skinner MK, Griswold MD. A sulfated glycoprotein synthesized by Sertoli cells and by epididymal cells is a component of the sperm membrane. Biol Reprod, 1984; 31: 1087-1101.
34. Sylvester SR, Morales C, Oko R, Griswold MD. Localization of sulfated glycoprotein-2 (clusterin) on spermatozoa and in the reproductive tract of the male rat. Biol Reprod, 1991; 45: 195-207.
35. Fischer-Colbrie R, Zangerle R, Frischenschlager I, Weber A, Winkler H. Isolation and immunological characterization of a glycoprotein from adrenal chromaffin granules. J Neurochem, 1984; 42: 1008-1016.

36. Fritz IB, Burdzy K, Setchell B, Blaschuk O. Ram rete testis fluid contains a protein (clusterin) which influences cell-cell interaction in vitro. Biol Reprod, 1983; 28:1173-1188.
37. Patzak A, Winkler H. Exocytotic exposure and recycling of membrane antigens of chromaffin granules: ultrastructural evaluation after immunolabeling. J Cell Biol, 1986; 102: 510-515.
38. Buttyan R, Olsson CA, Pintar J, Chang C, Bandyk M, Ng PY, et al. Induction of the TRPM-2 gene in cells undergoing programmed death. Mol Cell Biol, 1989; 9: 3473-3481.
39. Wilson MR, Easterbrook-Smith SB. Clusterin is a secreted mammalian chaperone. Trends Biochem Sci, 2000; 25: 95–98.
40. McLaughlin L, Zhu G, Mistry M, Ley-Ebert C, Stuart WD, Florio CJ, et al. Apolipoprotein J/clusterin limits the severity of murine autoimmune myocarditis. J Clin Invest, 2000; 106: 1105–1112.
41. Wehrli P, Charnay Y, Vallet P, Zhu G, Harmony J, Aronow B, et al. Inhibition of post-ischemic brain injury by clusterin overexpression. Nature Med, 2001; 7: 977–979.
42. Han BH, DeMattos RB, Dugan LL, Kim-Han JS, Brendza RP, Fryer JD, et al. Clusterin contributes to caspase-3-independent brain injury following neonatal hypoxia-ischemia. Nat Med, 2001; 7: 338–343.
43. Rosenberg ME, Girton R, Finkel D, Chmielewski D, Barrie A, Witte DP, et al. Apolipoprotein J/Clusterin Prevents a Progressive Glomerulopathy of Aging. Mol Cell Biol, 2002; 22: 1893–1902.
44. Michel D, Chatelain G, North S, Brun G. Stress-induced transcription of the clusterin/apoJ gene. Biochem J, 1997; 328: 45–50.
45. Rosenberg ME, Silkensen J. Clusterin and the kidney. Exp Nephrol, 1995; 3: 9-14.
46. Silkensen JR, Schwochau GB, Rosenberg ME. The role of clusterin in tissue injury. Biochem Cell Biol, 1994; 72: 483–488.
47. Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR. Clusterin has chaperone-like activity similar to that of small heat shock proteins. J Biol Chem, 1999; 274: 6875–6881.
48. Murphy BF, Saunders JR, O'Bryan MK, Kirszbaum L, Walker ID, d'Apice AJE. SP-40, 40 is an inhibitor of C5b-6-initiated haemolysis. Int Immunol, 1989; 1: 551-554.
49. Choi NH, Mazda T, Tomita M. A serum protein SP-40, 40 modulates the formation of membrane attack complex of complement on erythrocytes. Mol Immunol, 1989; 26: 835-840.

50. Patsch JR, Gotto AM, Olivecrona T, Eisenberg S. Formation of high density lipoprotein-like particles during lipolysis of very low density lipoproteins in vitro. Proc Natl Acad Sci USA, 1978; 75: 4519-4523.
51. Clay MA, Rye KA, Barter PJ. Evidence in vitro that hepatic lipase reduces the concentration of apolipoprotein A-I in rabbit high density lipoproteins. Biochim Biophys Acta, 1990; 1044: 50-56.
52. Ishikawa Y, Akasaka Y, Ishii T, Komiyama K, Masuda S, Asuwa N, et al. Distribution and synthesis of apolipoprotein J in the atherosclerotic aorta. Arterioscler Thromb Vasc Biol, 1998; 18: 665–672.
53. Baiersdorfer M, Schwarz M, Seehafer K, Lehman C, Heit A, Wagner H, et al. Toll-like receptor 3 mediates expression of clusterin/apolipoprotein J in vascular smooth muscle cells stimulated with RNA released from necrotic cells. Exp Cell Res, 2010; 316: 3489–3500.
54. Zhang H, Kim JK, Edwards CA, Xu Z, Taichman R, Wang CY. Clusterin inhibits apoptosis by interacting with activated Bax. Nat Cell Biol, 2005; 7: 909–915.
55. Poulakou M, Paraskevas KI, Wilson MR, Iliopoulos DC, Tsigris C, Michailidis D, et al. Apolipoprotein J and leptin levels in patients with coronary heart disease. In Vivo, 2008; 22: 537–542.
56. Ishikawa Y, Ishii T, Akasaka Y, Masuda T, Strong JP, Zieske AW, et al. Immunolocalization of apolipoproteins in aortic atherosclerosis in American youths and young adults: findings from the PDAY study. Atherosclerosis, 2001; 158(1): 215–225.
57. Schwarz M, Spath L, Lux CA, Paprotka K, Torzewski M, Dersch K, et al. Potential protective role of apoprotein J (clusterin) in atherogenesis: binding to enzymatically modified low-density lipoprotein reduces fatty acid-mediated cytotoxicity. Thromb Haemost, 2008; 100:110–118.
58. Wilson MR, Easterbrook-Smith SB. Clusterin binds by a multivalent mechanism to the Fc and Fab regions of IgG. Biochem Biophys Acta, 1992; 1159: 319–326.
59. French LE, Polla LL, Tschopp J, Schifferli JA. Membrane attack complex (MAC) deposits in skin are not always accompanied by S-protein and clusterin. J Investig Dermatol, 1992; 98:758–763.
60. Murphy BF, Davies DJ, Morrow W, d'Apice AJF. Localization of terminal complements components, S-protein and SP-40, 40 in renal biopsies. Pathology, 1989; 21:275–278.
61. Eddy AA, Fritz IB. Localization of clusterin in the epimembranous deposits of passive Heymann nephritis. Kidney Int, 1991; 39: 247–252.

62. Yamada K, Hori Y, Hanafusa N, Okuda T, Nagano N, Choi-Miura NH, et al. Clusterin is up-regulated in glomerular mesangial cells in complement-mediated injury. Kidney Int, 2001; 59: 137–146.
63. Newkirk MM, Apostolakos P, Neville C, Fortin PR. Systemic lupus erythematosus, a disease associated with low levels of clusterin/apoJ, an anti-inflammatory protein. J Rheumatol, 1999; 26: 597–603.
64. Poon S, Treweek TM, Wilson MR, Easterbrook-Smith SB, Carver J. Clusterin is an extracellular chaperone that specifically interacts with slowly aggregating proteins on their off-folding pathway. A FEBS Lett, 2002; 513: 259–266.
65. Diemer V, Hoyle M, Baglioni C, Millis AJT. Expression of porcine complement cytolysis inhibitor mRNA in cultured aortic smooth muscle cells. J Biol Chem, 1992; 267: 5257-5264.
66. French LE, Tschopp J, Schitferfi JA. Clusterin in renal tissue: preferential localization with the terminal complements complex and Ig deposits in glomeruli. Clin Exp Immunol, 1992; 88: 389-393.
67. Tschopp J, Choun A, Hertig-Schifer S, French LE. Clusterin, the human apolipoprotein and complement inhibitor binds to complement C7, C8β, and the b-domain of C9. J Immunol, 1993; 151(4): 2159-65.
68. James RW, Hochstrasser AC, Borghini I, Martin B, Pometta D, Hochstrasser D. Characterization of a human high density lipoprotein-associated protein, NA1/NA2. Arterioscler Thromb, 1991; 11:645-652.
69. Bandyk MG, Sawczuk IS, Olsson CA, Katz AE, Buttyan R. Characterization of the products of a gene expressed during androgen programmed cell death and their potential use as a marker of urogenital injury. J Urol, 1990; 143: 407-413.
70. Grima J, Zwain I, Lockshin RA, Bardin CW, Cheng CY. Diverse secretory patterns of clusterin by epididymis and prostate/seminal vesicles undergoing cell regression after orchiectomy. Endocrinology, 1990; 126: 2989-2997.
71. Sensibar JA, Griswold MD, Sylvester SR, Buttyan R, Bardin CW, Cheng CY, et al. Prostatic ductal system in rats: regional variation in localization of an androgen-repressed gene product, sulfated glycoprotein-2. Endocrinology, 1991; 128: 2091-2102.
72. Connor J, Buttyan R, Olsson CA, D'Agati V, O'Toole K, Sawczuk IS. SGP-2 expression as a genetic marker of progressive cellular pathology in experimental hydronephrosis. Kidney Int, 1991; 39: 1098-1103.

73. May PC, Lampert-Etchells M, Johnson SA, Poirier J, Masters JN, Finch CE. Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. Neuron, 1990; 5: 831-839.
74. French LE, Sappino AP, Tschopp J, Schifferli JA. Distinct sites of production and deposition of the putative cell death marker clusterin in the human thymus. J Clin Invest, 1992; 90: 1919-1925.
75. Michel D, Gillet G, Volovitch M, Pessac B, Calothy G, Brun G. Expression of a novel gene encoding a 51.5 kD precursor protein is induced by different retroviral oncogenes in quail neuroretinal cells. Oncogene Res, 1989; 4: 127-136.
76. Bova MP, Ding LL, Horwitz J, Fung BKK. Subunit Exchange of αA-Crystallin. J Biol Chem, 1997; 272, 29511–29517.
77. Trougakos IP, Gonos ES. Regulation of clusterin/apolipoprotein J, a functional homologue to the small heat shock proteins, by oxidative stress in ageing and age-related diseases. Free Radic Res, 2006; 40: 1324–1334.
78. Wilson MR, Yerbury JJ, Poon S. Potential roles of abundant extracellular chaperones in the control of amyloid formation and toxicity. Mol Biosyst, 2008; 4, 42–52.
79. Wyatt A, Yerbury J, Poon S, Dabbs R, Wilson MR. The chaperone action of Clusterin and its putative role in quality control of extracellular protein folding. Adv Cancer Res, 2009; 104*:* 89–114.
80. Wyatt AR, Yerbury JJ, Wilson MR. Structural Characterization of Clusterin-Chaperone Client Protein Complexes. J Biol Chem, 2009; 284*:* 21920–21927.
81. Poon S, Rybchyn MS, Easterbrook-Smith SB, Carver JA, Pankhurst GJ, Wilson MR. Mildly Acidic pH Activates the Extracellular Molecular Chaperone Clusterin. J Biol Chem, 2002; 277*:* 39532–39540.
82. Nuutinen T, Suuronen T, Kauppinen A, Salminen A. Clusterin: a forgotten player in Alzheimer's disease. Brain Res Rev, 2009; 61*:* 89–104.
83. Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet, 2009; 41*:* 1094–1099.
84. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere M et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet, 2009; 41*:* 1088–1093.

85. DeMattos RB, Cirrito JR, Parsadanian M, May PC, O'Dell MA, Taylor JW, et al. ApoE and clusterin cooperatively suppress Abeta levels and deposition: evidence that ApoE regulates extracellular Abeta metabolism in vivo. Neuron, 2004; 41*:* 193–202.
86. van den Berghe PV, Stapelbroek JM, Krieger E, de Bie P, van de Graaf SF, de Groot RE, et al. Reduced expression of ATP7B affected by Wilson disease-causing mutations is rescued by pharmacological folding chaperones 4-phenylbutyrate and curcumin. Hepatology, 2009; 50*:* 1783–1795.
87. Punnia-Moorthy A. Evaluation of pH changes in inflammation of the subcutaneous air pouch lining in the rat, induced by carrageenan, dextran and *Staphylococcus aureus*. J Oral Pathol, 1987; 16: 36–44.
88. Jacobus WE, Taylor GJT, Hollis DP, Nunnally RL. Phosphorus nuclear magnetic resonance of perfused working rat hearts. Nature, 1977; 265: 756–758.
89. Back T, Hoehn-Berlage M, Kohno K, Hossmann KA. Diffusion nuclear magnetic resonance imaging in experimental stroke. Correlation with cerebral metabolites. Stroke, 1994; 25: 494–500.
90. Yates CM, Butterworth J, Tennant MC, Gordon A. Enzyme activities in relation to pH and lactate in postmortem brain in Alzheimer-type and other dementias. J Neurochem, 1990; 55: 1624–1630.
91. Trougakos IP, Lourda M, Antonelou MH, Kletsas D, Gorgoulis VG, Papassideri IS et al. Intracellular clusterin inhibits mitochondrial apoptosis by suppressing p53-activating stress signals and stabilizing the cytosolic Ku70-Bax protein complex. Clin Cancer Res, 2009; 15: 48–59.
92. Reddy KB, Jin G, Karode MC, Harmony JA, Howe PH. Transforming growth factor beta (TGF beta)-induced nuclear localization of apolipoprotein J/clusterin in epithelial cells. Biochemistry, 1996; 35: 6157–6163.
93. Kang SW, Shin YJ, Shim YJ, Jeong SY, Park IS, Min BH, et al. Clusterin interacts with SCLIP (SCG10-like protein) and promotes neurite outgrowth of PC12 cells. Exp Cell Res, 2005; 309: 305–315.
94. D'Alessandro A, Righetti PG, Zolla L. The red blood cell proteome and interactome: an update. J Proteome Res 2010; 9: 144–163.
95. Carver JA, Lindner RA, Lyon C, Canet D, Dobson CM, Redfield C. The interaction of the molecular chaperone α-crystallin with unfolding α-lactalbumin: A structural and kinetic spectroscopic study. J Mol Biol, 2002; 318: 812–827.

96. Hochgrebe T, Pankhurst GJ, Wilce J, Easterbrook-Smith SB. pH-dependent changes in the in vitro ligand-binding properties and structure of human clusterin. Biochemistry, 2000; 39: 1411–1419.
97. Michel D, Chabot JG, Moyse E, Danik M, Quirion R. Possible functions of a new genetic marker in central nervous system: the sulfated glycoprotein-2 (SGP-2). Synapse, 1992; 11: 105-111.
98. May PC, Finch CE. Sulfated glycoprotein 2: new relationships of this multifunctional protein to neurodegeneration. Trends Neurosci, 1992; 15: 391-396.
99. Danik M, Chabot JG, Hassan-Gonzalez D, Suh M, Quirion R. Localization of sulfated glycoprotein-2/clusterin mRNA in the rat brain by in situ hybridization. J Comp Neurol, 1993; 334: 209-227.
100. Lampertetchells M, McNeil TH, Laping NJ, Zarow C, Finch CE, May PC. Sulfated glycoprotein-2 is increased in rat hippocampus following entorhinal cortex lesioning. Brain Res, 1991; 563: 101-106.
101. Pasinetti GM, Cheng HW, Moran DG, Lampertetchells M, Mcneill TH, Finch CE. Astrocytic messenger RNA responses to striatal deafferentation in male rat. Neuroscience, 1993; 53: 199-211.
102. Duguid JR, Bohmont CW, Liu CW, Tourtelotte WW. Changes in brain gene expression shared by scrapie and Alzheimer disease. Proc Nat Acad Sci USA, 1989; 86: 7260-7264.
103. Danik M, Chabot JG, Mercier C, Benabib AL, Chauvin C, Quirion R, Suh M. Human gliomas and epileptic foci express high levels of a mRNA related to rat testicular sulfated glycoprotein 2, a purported marker of cell death. Proc Nat Acad Sci USA, 1991; 88: 8577-8581.
104. Jones SE, Meerabux JMA, Yeats DA, Neal MJ. Analysis of differentially expressed genes in retinitis-pigmentosa retinas–Altered expression of clusterin messenger RNA. FEBS Lett, 1992; 300: 279-282.
105. Wong P, Borst DE, Farber D, Danciger JS, Tenniswood M, Chader GJ, et al. Increased TRPM-2/clusterin mRNA levels during the time of retinal degeneration in mouse models of retinitis pigmentosa. Biochem Cell Biol, 1994; 72: 439-446.
106. Norman DJ, Feng L, Cheng SS, Gubbay J, Chan E, Heintz N. The lurcher gene induces apoptotic death in cerebellar Purkinje cells. Development, 1995; 121: 1183-1193.
107. McGeer PL, Kawamata T, Walker DG. Distribution of clusterin in Alzheimer brain tissue. Brain Res, 1992; 579: 337-341.

108. Choi-Miura NH, Khara Y, Fukuchi K, Takeda M, Nakano Y, Tobe T, et al. SP-40, 40 is a constituent of Alzheimer' amyloid. Acta Neuropathol, 1992; 83: 260-264.
109. Matsubara E, Frangione B, Ghiso J. Characterization of apolipoprotein J-Alzheimer's A beta interaction. J Biol Chem, 1995; 270(13): 7563-7567.
110. Yasuhara O, Aimi Y, Yamda T, Matsuo A, McGeer EG, McGeer PL. Clusterin as a marker for ischaemic Purkinje cells in human brain. Neurodegeneration, 1994; 3: 325-329.
111. Fritz IB, Murphy B. Clusterin: Insights into a multifunctional protein. Trends Endocrinol Metab, 1993; 4: 41-45.
112. Jordan-Starck TC, Witte DP, Aronow BJ, Harmony JAK. Apolipoprotein J: A membrane policeman? Curr Op Lipidol, 1992; 3: 75-85.
113. Rosenberg ME, Dvergsten J, Correa-Rotter R. Clusterin: An enigmatic protein recruited by diverse stimuli. J Lab C/in Med, 1993; 121: 205-214.
114. Correa-Rotter R, Nath KA, Hostetter TH, Rosenberg ME. Clusterin expression in acute and chronic oxidative renal injury, (abstract). J Am Soc Nephrol, 1991; 2: 660.
115. Correa-Rotter R, Hostetter TH, Manivel JC, Eddy AA, Rosenberg ME. Intrarenal distribution of clusterin following reduction of renal mass. Kidney Int, 1992; 41: 938-950.
116. Harding MA, Chadwick U, Gattone VH II, Calvet JP. The SGP-2 gene is developmentally regulated in the mouse kidney and abnormally expressed in collecting duct cysts in polycystic kidney disease. Dev Biol, 1991; 146: 483-490.
117. Kissinger C, Skinner MK, Griswold MD. Analysis of Sertoli cell-secreted proteins by two-dimensional gel electrophoresis. Biol Reprod, 1982; 27: 233–240.
118. Griswold MD, Morales C, Sylvester SR. Molecular biology of the Sertoli cell. Oxf Rev Reprod Biol, 1988; 10: 124–161.
119. Leger JG, Montpetit ML, Tenniswood MP. Characterization and cloning of androgen-repressed mRNAs from rat ventral prostate. Biochem Biophys Res Commun, 1987; 147: 196–203.
120. Sensibar JA, Qian Y, Griswold MD, Sylvester SR, Bardin CW, Cheng CY, et al. Localization and molecular heterogeneity of sulfated glycoprotein-2 (clusterin) among ventral prostate, seminal vesicle, testis, and epididymis of rats. Biol Reprod 1993; 49: 233–242.
121. Sylvester SR, Griswold MD. 1995. The reproductive biology of clusterin. In: Harmony JAK (Ed.). Clusterin: Role in Vertebrate Development, Function and Adaption. RG Landes, Georgetown; TX: 1995, pp. 141-163.