Oxidative Stress in Malignant Progression: The Role of Clusterin, A Sensitive Cellular Biosensor of Free Radicals

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Clusterin/Apolipoprotein J (CLU) gene is expressed in most human tissues and encodes for two protein isoforms; a conventional heterodimeric secreted glycoprotein and a truncated nuclear form. CLU has been functionally implicated in several physiological processes as well as in many pathological conditions including ageing, diabetes, atherosclerosis, degenerative diseases, and tumorigenesis. A major link of all these, otherwise unrelated, diseases is that they are characterized by increased oxidative injury due to impaired balance between production and disposal of reactive oxygen or nitrogen species. Besides the aforementioned diseases, CLU gene is differentially regulated by a wide variety of stimuli which may also promote the production of reactive species including cytokines, interleukins, growth factors, heat shock, radiation, oxidants, and chemotherapeutic drugs. Although at low concentration reactive species may contribute to normal cell signaling and homeostasis, at increased amounts they promote genomic instability, chronic inflammation, lipid oxidation, and amorphous aggregation of target proteins predisposing thus cells for carcinogenesis or other age-related disorders. CLU seems to intervene to these processes due to its small heat-shock protein-like chaperone activity being demonstrated by its property to inhibit protein aggregation and precipitation, a main feature of oxidant injury. The combined presence of many potential

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regulatory elements in the CLU gene promoter, including a Heat-Shock Transcription Factor-1 and an Activator Protein-1 element, indicates that CLU gene is an extremely sensitive cellular biosensor of even minute alterations in the cellular oxidative load. This review focuses on CLU regulation by oxidative injury that is the common molecular link of most, if not all, pathological conditions where CLU has been functionally implicated.

I. INTRODUCTION

The conventional isoform of the human Clusterin (CLU) gene (NCBI: NM_203339.1/NP_976084.1, CLU isoform 2) encodes for a ubiquitously expressed secreted protein of ~75 kDa (sCLU) (Wong *et al.*, 1994) being involved in several physiological processes including lipid transportation in the serum (de Silva *et al.*, 1990), development, and differentiation (Aronow *et al.*, 1993; Jenne and Tschopp, 1989; Trougakos *et al.*, 2006b) (see also chapter "Cell Protective Functions of Secretory CLU"). In certain cell types, apoptosis induction correlates with the appearance of alternative CLU protein isoforms which mostly localize in cell nucleus (nCLU). nCLU can be produced by either alternative splicing or internal translation initiation; in both cases, the N-terminal sequence of sCLU that encodes the hydrophobic endoplasmic reticulum (ER) targeting peptide is bypassed resulting in cytosolic or nuclear protein localization (Leskov *et al.*, 2003; Yang *et al.*, 2000). Almost invariably nCLU exerts a cytostatic and/or proapoptotic function (Leskov *et al.*, 2003; Moretti *et al.*, 2007; Yang *et al.*, 2000) (see also chapter "Nuclear CLU (nCLU) and the Fate of the Cell").

Two main functions have been assigned to sCLU up to date. The one refers to its Apolipoprotein function at the high-density lipoprotein (HDL) particles (de Silva *et al.*, 1990) and the second to its ability to bind to hydrophobic regions of partially unfolded proteins and via an ATP-independent mechanism to inhibit protein aggregation and precipitation otherwise caused by physical or chemical stresses (e.g., heat, reduction) (Humphreys *et al.*, 1999; Poon *et al.*, 2000). On the basis of this latter property, sCLU was classified as an extracellular functional homologue of the small heat-shock proteins (sHSPs) (Wilson and Easterbrook-Smith, 2000) (see also chapter "The Chaperone Action of CLU and Its Putative Role in Quality Control of Extracellular Protein Folding"). Interestingly, it seems that in certain cell types sCLU can escape the secretory pathway and localize in the nucleocytosolic continuum and mitochondria (Nizard *et al.*, 2007; Trougakos *et al.*, 2009b; Zhang *et al.*, 2005) indicating that sCLU may also function as an intracellular chaperone.

The CLU gene promoter is highly conserved and contains several regulatory elements that regulate the complex tissue-specific control of the gene.

Among these elements are a conserved 14-bp "CLU-specific element" (CLE), which is recognized by the Heat-Shock Transcription Factor 1 (HSF1) and a site for the Activator Protein-1 (AP-1) (Jin and Howe, 1997; Michel *et al.*, 1995; Wong *et al.*, 1994). By acting in synergy, the CLE and AP-1 elements can make the CLU gene promoter particularly sensitive to even minute environmental changes or alterations in the cellular oxidative load. In support, the CLU gene is responsive to a wide variety of both intrinsic or extrinsic factors including oncogenes, transcription and growth factors, cytokines and most stress-inducing agents including oxidants, hyperoxia, proteotoxic stress, heavy metals, UVA, UVB, ionizing radiation (IR), heat shock, and chemotherapeutic drugs (Calero *et al.*, 2005; Klokov *et al.*, 2004; Trougakos and Gonos, 2002, 2006) (see also chapter "Regulation of CLU Gene Expression by Oncogenes, Epigenetic Regulation, and the Role of CLU in Tumorigenesis," Vol. 105). Moreover, CLU is differentially regulated and have been functionally involved in a wide range of distinct pathological conditions including atherosclerosis, diabetes, kidney, and neuron degeneration as well as carcinogenesis (Trougakos and Gonos, 2006); in most, if not all, of these pathologies differential regulation referred to sCLU.

The only common characteristic shared by all these, otherwise unrelated in their etiology and/or clinical manifestation, pathological conditions is the fact that they are characterized by increased oxidative stress and injury, which may then promote proteotoxic stress, genomic instability, arrest of proliferation, and high rates of cellular death. In the current review, we promote the idea that sCLU is a sensitive cellular biosensor of oxidative stress that functions to protect cells from the deleterious effects of free radicals and their derivatives.

II. MOLECULAR EFFECTS OF ALTERED OXIDATIVE LOAD IN HUMAN CELLS

Oxygen is essential to support aerobic metabolism and life. However, since the normal cellular milieu is reductive, oxygen molecules undergo univalent reductions forming oxygen-free radicals (Beckman and Ames, 1998). In the last two decades there has been an significant interest in the role of oxygen-free radicals, more generally known as "reactive oxygen species" (ROS) and "reactive nitrogen species" (RNS) in experimental and clinical medicine (Dröge, 2003). Oxygen-free radicals can be produced by both endogenous and exogenous sources. Endogenously oxygen-free radicals may arise as by-products of normal aerobic metabolism (i.e., excessive stimulation of NAD(P)H oxidases, oxidative energy metabolism, P450 metabolism, and peroxisomes), as second messengers in various

signal transduction pathways or during inflammatory neutrophils and macrophage cells activation. Exogenous sources of free radical production are various types of radiations (e.g., UV light, X-rays, or gamma rays), metal-catalyzed reactions, and atmospheric pollutants (e.g., chlorinated compounds) (Dröge, 2002; Martindale and Holbrook, 2002; Valko *et al.*, 2006).

Oxidative stress occurs due to overproduction of oxygen-free radicals that exceeds the cell's antioxidant capacity (Klaunig and Kamendulis, 2004). Oxygen-free radicals, whether produced endogenously as a consequence of normal cell functions or derived from external sources, pose a constant threat to cells living in an aerobic environment as they can severely damage DNA, proteins, and lipids (Martindale and Holbrook, 2002). More that 100 oxidative DNA adducts have been identified (Demple and Harrison, 1994) and in a given cell, an estimated 10^5 oxidative lesions per day are formed (Fraga *et al.*, 1990). Cells contain a number of antioxidant defenses (either nonenzymatic antioxidants or antioxidant enzymes) to minimize fluctuations in oxygen-free radicals. Nonenzymatic antioxidants include vitamins E and C, natural flavonoids, carotenoids, thiol antioxidants (glutathione, thioredoxin, and lipoic acid), melatonin, and other compounds (McCall and Frei, 1999), while enzymatic antioxidants include catalase, glutathione peroxidases, and superoxide dismutase (SOD) (Mates *et al.*, 1999). Host survival depends upon the ability of cells and tissues to adapt to or resist the stress, and repair or remove damaged molecules or cells. Numerous stress response mechanisms have evolved for these purposes and they are rapidly activated in response to oxidative insults (Valko *et al.*, 2006).

Oxygen-free radicals are well recognized for playing a dual role as both beneficial and deleterious species. More specifically, low physiological amounts of oxidants may act as a secondary messenger in intracellular signaling cascades promoting normal cell homeostasis and proliferation. Abnormally high levels of ROS may, however, result in deregulation of redox-sensitive signaling pathways and extensive cell damage leading to responses which may include arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, reversible growth arrest, senescence, or cellular death (Fig. 1) (Storz, 2005; Valko *et al.*, 2006).

Cellular damage due to increased oxidative stress may be reflected to DNA, proteins, and lipids. DNA damage is mainly induced via the formation of 8-hydroxy-2′-deoxyguanosine (8-oxodG) that is a hydroxyl radical-damaged guanine nucleotide. Additional effects may relate to single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA cross-links (Floyd *et al.*, 1990). Protein modifications relate to direct protein oxidation (e.g., at cysteine residues), formation of peroxyl radicals (Stadtman, 1992), conjugation with lipid peroxidation products, glycation, and glycoxidation (Chondrogianni and Gonos, 2005;

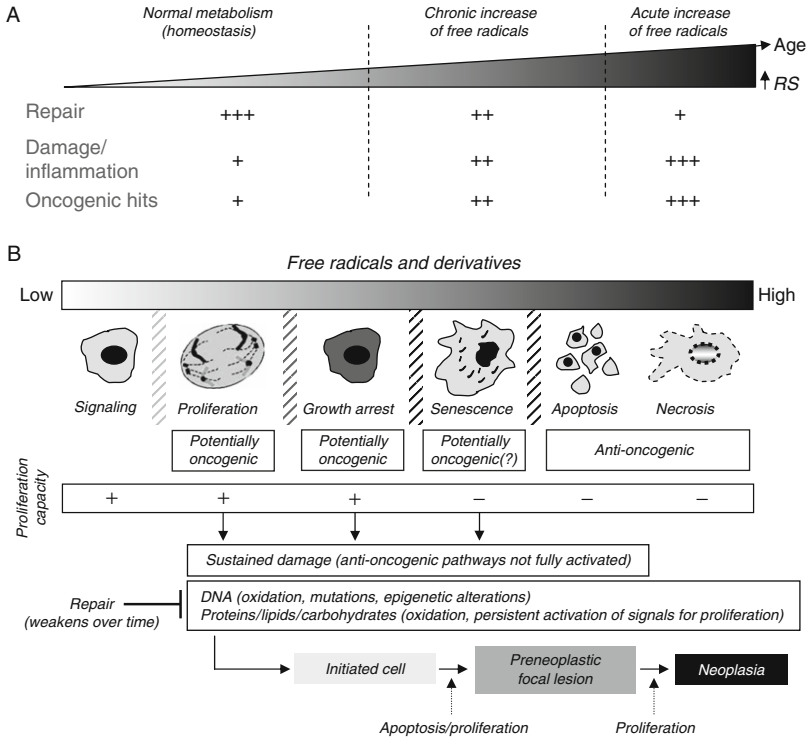

Fig. 1 Oxidative stress and cancer. (A) During ageing the load of oxidants in the organism gradually increases, due to both intrinsic and extrinsic factors, resulting in the accumulation of damaged molecules (e.g., nucleic acids, proteins, lipids, etc.), the impairment of the cell signaling regulatory pathways, and consequently the increase of the oncogenic hits. Thus, these effects directly relate to age-related diseases including cancer. The process can be significantly accelerated by various factors including several metabolic disorders (e.g., diabetes, obesity, etc.) or unhealthy habits (e.g., smoking, prolonged exposure to sun, etc.). (B) At the cellular level free radicals are not always deleterious as at low nonhazardous amounts contribute to physiological signaling and homeostasis. However, as the intracellular and/or extracellular amounts of free radicals and their derivatives increase so does the damage resulting in various responses which may include growth arrest, premature senescence, apoptosis, or necrosis. Although some are clearly tumor suppressing states (e.g., apoptosis and necrosis) others maybe potentially oncogenic as they combine gradual damage accumulation with a retained ability for proliferation. As the repair mechanisms weaken over time (because they are also targets of oxidative damage) more damaged molecules accumulate. This array of events increases dramatically the chances for the appearance of tumor initiating cells which may then give rise to preneoplastic focal lesions and eventually to neoplasia. CLU has been functionally implicated in all cellular states or responses described.

Poppek and Grune, 2006). These modifications may lead to loss of function, gain of function, or switch to a different function. Finally, lipid alterations mainly refer to lipid peroxidation (Yu and Chung, 2006).

Oxidative stress regulates an immense array of distinct signaling pathways that conclude in the activation of several transcription factors including, AP-1, nuclear factor-kappa beta (NF-κB), HSF1, p53, and signal transducer and activator of transcription (STAT) (Martindale and Holbrook, 2002; Storz, 2005). AP-1 is a collection of dimeric basic region-leucine zipper proteins that belong to the Jun, Fos, Maf, and ATF subfamilies (Chinenov and Kerppola, 2001). AP-1 activity is induced by H_2O_2 as well as from several cytokines and other physical and chemical stresses (Klatt *et al.*, 1999). Activation of AP-1 mostly results in increased cell proliferation (Shaulian and Karin, 2001), while AP-1 proteins may also participate in oncogenic transformation by interacting with activated oncogenes such as H-Ras (Schutte *et al.*, 1989). NF-κB is a ubiquitously expressed inducible transcription factor which in its active form is a dimer consisting of proteins from the Rel family, namely p50, p52, c-Rel, v-Rel, p65, and Rel B (Chen *et al.*, 2001). NF-κB regulates a wide array of different genes involved in immune function, inflammation, stress response, differentiation, apoptosis, cell survival, and growth. Involvement of NF-κB in tumor initiation and progression mostly occurs in tissues in which cancer-related inflammation typically occurs (such as the gastrointestinal tract and the liver), while recent studies have shown that NF-κB signaling may also play critical roles in the epithelial-to-mesenchymal transition (EMT) and cancer stem cells physiology (Sarkar *et al.*, 2008). Activated NF-κB, functions together with other transcription factors, such as AP-1 to regulate the expression of immune and inflammatory genes including interleukins (IL)-2, -2R, -6, and -8 and tumor necrosis factor-alpha (TNF-α). Most of these proteins can also cause the activation of NF-κB, forming a positive regulatory loop to amplify inflammatory responses (Chen *et al.*, 2001; Sarkar *et al.*, 2008). HSF1, under normal conditions is kept inactive in the cytosol by mainly binding to HSP90 (Morimoto, 2002). Upon stress, the suppressing chaperones become occupied by the misfolded proteins liberating thus HSF1 which can then translocate to the nucleus and activate several target genes (Morimoto, 2002; Soti *et al.*, 2005). P53, the so-called "genome guardian" (Lane, 1992), is critically involved in the processes of many different stress responses and it is disrupted in more than half of all human cancers (Vousden and Lane, 2007). Besides the aforementioned nuclear transcription factors, free radicals can also regulate a wide panel of receptor tyrosine kinases, nonreceptor protein kinases, the H-Ras oncogene, protein tyrosine phosphatases, and serine/threonine kinases [e.g., transforming growth factor-beta (TGF-β)] (Valko *et al.*, 2006).

III. OXIDATIVE INJURY IN AGEING AND AGE-RELATED DISEASES

Ageing is an inevitable consequence of life for nearly all organisms. It mostly reflects the outcome of complicated interactions between genetic factors along with the accumulation of a variety of deleterious stochastic changes over time due to a progressive failure of homeostasis that promotes multiple biochemical, molecular, and cellular changes which lead to increased disability, morbidity, and inevitably to death (Kirkwood, 2002). Thus, ageing is an adaptive process, not caused by a single factor or process, but it is rather a multifactorial procedure modulated by the interplay among genetic and environmental factors (Lane, 2003).

According to the "free radical theory of ageing" because of their high reactivity, oxygen-free radicals would lead to unavoidable and potentially deleterious by-products. Such an increasingly damaging process could be responsible for degenerative diseases and ageing (Beckman and Ames, 1998). Thus, it is generally accepted that oxidative stress is a major risk in ageing (Finkel and Holbrook, 2000; Golden *et al.*, 2002). In support, increased levels of oxidative and proteotoxic stress, DNA damage along with progressive changes in free radical-mediated regulatory processes have been linked to *in vivo* ageing in numerous reports (Carrard *et al.*, 2002; Grune *et al.*, 2004; Mariani *et al.*, 2005).

In invertebrates, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, several mutants which reduce damage by oxidants result in increased life span providing thus a correlation between oxidative stress, life span, and genetic components (Hekimi and Guarente, 2003). Similarly, in mammals, mutation of the $p66^{SHC}$ protein increased resistance to oxidative stress and prolonged life span (Migliaccio *et al.*, 1999). The level of $p66^{SHC}$ decreased with advanced age (Sagi *et al.*, 2005) and ROS-mediated dysfunction in aortic endothelial cells was prevented in $p66^{SHC-/-}$ mice (Francia *et al.*, 2004). ROS implication in human ageing is also supported by *in vitro* studies at various cellular models. Specifically, normal human cells undergo a limited number of divisions in culture and progressively reach a state of irreversible growth arrest, a process termed as replicative senescence (RS). RS occurs because human cells (due to the absence of telomerase activity and owing to the biochemistry of DNA replication) acquire one or more critically short telomere (Campisi, 2005). Interestingly, cells can also senesce prematurely having long telomeres if exposed to a wide range of distinct types of stress including oxidative stress, proteotoxic stress, DNA damage, cytotoxic drugs, strong mitogenic signals, and disrupted chromatin (Collado and Serrano, 2006). This process is called stress-induced premature senescence (SIPS) and is phenotypically and biochemically similar to RS. Independently of the

triggering event, *in vitro* senescing cells are characterized by accumulation of ROS, reduced proteasome activity, and high rates of protein oxidation/-carbonylation (Chondrogianni and Gonos, 2005; Trougakos *et al.*, 2002a).

Parallel to its continuous effects during life span there is growing evidence implicating oxidative damage to DNA, proteins, and lipids in the pathogenesis of various diseases. More specifically, oxidative stress plays a central role in various pathological conditions such as atherosclerosis, diabetes, neurodegeneration, inflammation, rheumatoid arthritis, and cancer (Dröge, 2002; Grune *et al.*, 2004; Mariani *et al.*, 2005; Sarkar *et al.*, 2008).

Increased production of ROS has been related to the risk for cardiovascular diseases such as atherosclerosis, angina, and myocardial infarction (Yoshizumi *et al.*, 2001). In fact, atherosclerosis represents a state of increased oxidative stress characterized by early lipid and protein oxidation in the vascular wall either at cardiovascular or cerebrovascular diseases (Stocker and Keaney, 2004). In support, heart failure associates with oxidative stress in animal studies, while during congestive heart failure the activation of most of the neurohormonal and inflammatory signaling pathways induce oxidative stress through several mechanisms including angiotensin II, aldosterone, TNF-α, and proinflammatory cytokines (Hill and Singal, 1997; Li *et al.*, 1998). ROS accumulation in pancreas is believed to play a central role in beta cells death and type I diabetes progression which is characterized by the severe destruction of insulin-producing beta cells (Ho and Bray, 1999). Most signal transduction responses due to pancreas-specific ROS production are mediated by activated NF-κB (Mollah *et al.*, 2008). Brain aging is associated with a progressive imbalance between antioxidant defenses and intracellular concentrations of oxygen-free radicals as exemplified by increases in products of DNA, protein, and lipid oxidation (Dröge and Schipper, 2007). Several lines of evidence highlight ROS implication in neurodegenerative diseases. Both brain ischemia and the condition of ischemia/reperfusion occurring after stroke, has been associated with free radical-mediated reactions (Alexandrova *et al.*, 2004) while most studies agree that oxidative stress contributes to dopaminergic cell degeneration in Parkinson's disease (Jenner, 2003) and that oxidative stress is one of the earliest events in Alzheimer's disease (AD) (Zhu *et al.*, 2004). Administration of vitamin E leads to a slowing of the AD progression and the patients taking antioxidant vitamins and anti-inflammatory compounds have a lower incidence of AD (Mancuso *et al.*, 2007). Oxidizing conditions cause protein cross-linking and aggregation of Aβ peptides (Dyrks *et al.*, 1993) and also contribute to aggregation of tau and other cytoskeletal proteins (Bellomo and Mirabelli, 1992). Finally, ROS seem to contribute significantly to tissue injury and inflammation in rheumatoid arthritis and the control of inflammation in arthritic patients by antioxidants could become an efficient strategy for antirheumatic therapy (Bauerova and Bezek, 1999).

IV. OXIDATIVE STRESS IN MALIGNANT PROGRESSION

Cancer development is a multifactorial, multistage process characterized by the cumulative action of additive events occurring in a single cell. It is generally described by three stages: initiation, promotion, and progression. Mutations, oncogene activation, genomic instability, and epigenetic factors deregulate gene expression profiles and disrupt the molecular networks providing tumor cells with sufficient diversity and proliferative advantage to evolve in the healthy tissue and eventually to metastasize (Halazonetis *et al.*, 2008; Weinberg, 2008).

Oxygen-free radicals are potential carcinogens because they can facilitate all three stages of tumor formation including mutagenesis, tumor promotion, and progression (Dröge, 2002; Halliwell, 2007; Klaunig and Kamendulis, 2004; Valko *et al.*, 2006). During initiation, a nonlethal mutation in DNA is followed by at least one round of DNA synthesis to fix the damage (e.g., due to 8-oxodG) and produce an altered cell. The promotion stage requires the continuous presence of the tumor promotion stimulus and therefore it is a reversible process. This stage may eventually result in the formation of an identifiable focal lesion which is characterized by the clonal expansion of finding cells via the induction of cell proliferation and/or inhibition of apoptosis. Production of ROS during this stage of carcinogenesis is the main line of ROS-related protumor effect as many carcinogens have a strong inhibiting effect on cellular antioxidant defense systems such as SOD, catalase, and glutathione. Low sustained level of oxidative stress in this state may also stimulate cell division resulting thus in tumor growth (Dreher and Junod, 1996). The final stage of the carcinogenic process is progression. This stage involves cellular and molecular changes that occur from the preneoplastic to the neoplastic state and is irreversible as it includes accumulation of additional genetic damage, genetic instability, and angiogenesis (Carmeliet, 2000).

Increased levels of oxidative DNA lesions (8-oxodG) have been noted in various tumors, strongly implicating ROS involvement in the etiology of cancer (Loft and Poulsen, 1996), while deficiency of various antioxidant defense enzymes raises oxidative damage levels and promotes age-related cancer development in animals (Elchuri *et al.*, 2005). The interaction of ROS with DNA has been reported to result in both fragmentation and strand breaks, as well as in decreased DNA methylation (Weitzman *et al.*, 1994). Additional evidence has implicated oxygen-free radicals and mitochondrial oxidative DNA damage in the carcinogenesis process (Cavalli and Liang, 1998). Aside from oxidized nucleic acids, other oxidation-derived DNA adducts that appear important in chemical carcinogenesis are those produced due to damage in cellular biomembranes that result in lipid

peroxidation, a process that generates a variety of mutagenic products (Janero, 1990). As mentioned, cell exposure to nontoxic concentrations of ROS may result in aberrant increase of cell proliferation, survival, and cellular migration. These effects mostly relate to activation of AP-1 and NF-κB signal transduction pathways (Valko *et al.*, 2006). The induction of oxidative stress modulates the function of several cell-cycle checkpoints (Shackelford *et al.*, 2000) and may also inhibit intercellular communication (Upham *et al.*, 1997) allowing the preneoplastic cell to escape the growth control of normal surrounding cells. Finally, recent data showed that the tumor suppressor genes $p15^{INK4B}$ and $p16^{INK4A}$ are among the major target genes in oxidative stress-induced carcinogenesis (Toyokuni, 2008). Therefore, there may be "fragile" sites in the genome that are more susceptible to oxidative stress.

Beyond the effects of oxygen-free radicals in tumorigenesis, the cancer cells are under increased and persistent oxidative stress. This elevated intrinsic oxygen-free radicals-related stress in cancer cells mostly relate to oncogenic stimulation, increased metabolic activity, mitochondrial malfunction, and low levels of enzymes such as SOD, glutathione peroxidase, glutathione reductase, and catalase (Gupte and Mumper, 2009). High endogenous levels of oxygen-free radicals have been found in human colorectal carcinoma (Kondo *et al.*, 1999), in various types of leukemia (Devi *et al.*, 2000) as well as in breast (Yeh *et al.*, 2005), ovarian (Senthil *et al.*, 2004), and stomach cancer (Batchioglu *et al.*, 2006). Moreover, ROS levels in cancer cells relate to tumor aggressiveness as it has been reported that the difference in the redox status of two prostrate cancer cell lines correlated with the degree of their aggressiveness (Chaiswing *et al.*, 2007).

Inflammatory process induces oxidative stress and reduces cellular antioxidant capacity. Chronic inflammation is a pathological condition characterized by continued active inflammation response and tissue destruction. In some types of cancer, inflammatory conditions are present before a malignant change occurs, while in others, an oncogenic change induces an inflammatory microenvironment that accelerates the development of tumors. The triggers of chronic inflammation that may increase cancer risk include microbial infections (i.e., *Helicobacter pylori* for gastric cancer and mucosal lymphoma), autoimmune diseases (i.e., inflammatory bowel disease for colon cancer), and cryptogenic inflammatory conditions (i.e., prostatitis for prostate cancer) (Mantovani *et al.*, 2008). Particularly, regarding prostate cancer, there is emerging evidence that prostatic inflammation may contribute to prostate growth by introducing hyperplastic or neoplastic changes (Minelli *et al.*, 2009; Sciarra *et al.*, 2008). Pancreatic inflammation, mediated by cytokines, ROS, and upregulated proinflammatory pathways, may play a key role in the early development of pancreatic malignancy (Farrow and Evers, 2002), while epidemiological data have consistently

demonstrated a positive relation between obesity and colorectal malignancy (Gunter and Leitzmann, 2006). Moreover, B lymphocytes are required for establishing chronic inflammatory states that promote *de novo* epithelial carcinogenesis (de Visser *et al.*, 2005).

The main substances that link inflammation to cancer via oxidative/nitrosative stress are key intrinsic agents such as transcription factors (e.g., NF-κB and STAT3) and major inflammatory cytokines (e.g., IL-1β, IL-6, IL-23, and TNF-α) which lie downstream of NF-κB, the "master" gene transcription factor for promoting inflammation (Sarkar *et al.*, 2008). Further, the inflammatory mediators such as cytokines, prostaglandins, and growth factors, which are regulated by NF-κB, can induce genetic and epigenetic changes including mutations in tumor suppressor genes, DNA methylation, and post-translational modifications, leading to the development and progression of cancer (Federico *et al.*, 2007).

On the basis of these facts it was recently proposed that inflammation may constitute the seventh hallmark that collectively dictate malignant growth (Mantovani, 2009); the other six being, self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg, 2000).

V. CLUSTERIN AS A SENSITIVE CELLULAR BIOSENSOR OF OXIDATIVE STRESS

A. Clusterin Regulation and Function at the Cellular Level as a Response to Free Radicals and Their Derivatives

Considering that oxidants and oxidation injury modulate the activity of both the AP-1 and HSF1 transcription factors (Martindale and Holbrook, 2002) the combinatorial presence of both the AP-1 and CLE regulatory elements in the CLU gene promoter (Jin and Howe, 1997; Michel *et al.*, 1995) should make CLU gene an extremely sensitive biosensor to environmental changes and particularly to the direct or indirect downstream effects of free radicals and their derivatives.

Indeed, both sCLU (at low nontoxic doses) and nCLU (at high doses) are induced in human cells by IR, a potent inducer of ROS accumulation and genomic instability (Criswell *et al.*, 2005; Leskov *et al.*, 2003). Moreover, photooxidative cell damage in human cultured retinal pigmented epithelium upregulated nCLU (Ricci *et al.*, 2007). Proteotoxic stress induction due to proteasome inhibition results in sCLU mRNA upregulation via HSF1 and

HSF2 binding to the CLE element (Balantinou *et al.*, 2009; Loison *et al.*, 2006). Many oxidants (e.g., ethanol, tert-butylhydroperoxide, or H_2O_2) induce an immediate sCLU mRNA and protein upregulation in human diploid fibroblasts (Frippiat *et al.*, 2001), while CLU was found to be induced in retinal pigment epithelial cells after subtoxic oxidative stress via TGF-β release (Yu *et al.*, 2009) and in human neuroblastoma cells under conditions of increased production of ROS and lipid peroxidation (Strocchi *et al.*, 2006). At *in vivo* models both the organochlorine pesticides Methoxychlor and Lindane when administered in rats promoted a sequential reduction in the activities of catalase and SOD with concomitant increase in the levels of lipid peroxidation, HSP70, and sCLU (Saradha *et al.*, 2008; Vaithinathan *et al.*, 2009). A direct correlation of sCLU expression levels and organismal oxidative stress in also evident by the findings that sCLU concentration in human serum correlated positively with exposure to acute *in vivo* oxidative stress induced by heavy metals (Alexopoulos *et al.*, 2008), while, on the other hand, adult-onset of caloric restriction that retarded age-related oxidative damages resulted in CLU suppression in kidneys of rats (Chen *et al.*, 2008c). Finally, immunohistochemical analysis showed that CLU is constantly associated with altered elastic fibers in aged human skin and elastotic material of sun-damaged skin. It was found that CLU binds elastin, and effectively inhibited UV-induced aggregation of elastin by exerting its chaperone activity(Janig *et al.*, 2007).

Several studies demonstrate that sCLU suppress the deleterious effects of oxidants. More specifically, sCLU protects human epidermoid cancer cells from H_2O_2, superoxide anion, hyperoxia, and UVA (Schwochau *et al.*, 1998; Viard *et al.*, 1999); normal human fibroblasts from cytotoxicity induced by ethanol, tert-butylhydroperoxide, or UVB (Dumont *et al.*, 2002); human prostate cancer cells from oxidative stress-induced DNA damage (Miyake *et al.*, 2004); lung fibroblasts from cigarette smoke oxidants (Carnevali *et al.*, 2006); and human retinal cells from free-radical damage (Kim *et al.*, 2007a) or *in vitro* ischemia (Kim *et al.*, 2007b). Taken together these data clearly indicate the sensitivity of CLU gene promoter to alterations in the cell oxidative load and the cytoprotective role of sCLU in oxidative injury.

As it is shown in Fig. 1, oxidative stress in normal human cells can trigger a wide range of responses including proliferation, reversible growth arrest, senescence, or cellular death. Interestingly, CLU has been implicated in all these cellular states and processes.

More specifically, in certain cellular contexts sCLU or nCLU transgene-mediated overexpression has been reported to induce growth retardation at the G_2/M checkpoint, reduce DNA synthesis, and augment the cytostatic effect of genotoxic stress (Bettuzzi *et al.*, 2002; Scaltriti *et al.*, 2004b; Thomas-Tikhonenko *et al.*, 2004; Trougakos *et al.*, 2005) while

extracellular CLU blocked cellular proliferation in prostate cancer cells (Zhou *et al.*, 2002). However, although high CLU levels may reduce the cycling potential of human cells for a short period of time, these cells eventually adapt to high CLU levels and continue cycling (Bettuzzi *et al.*, 2002; Trougakos *et al.*, 2005). Interestingly, exogenous sCLU promoted growth in astrocytes by activating epidermal growth factor receptor and through the Ras/Raf-1/MEK/ERK signaling cascade (Shim *et al.*, 2009). sCLU is a faithful biomarker of cellular senescence as it is upregulated during RS (Petropoulou *et al.*, 2001) or during SIPS induction by UVB (Debacq-Chainiaux *et al.*, 2005), subcytotoxic doses of H_2O_2 (Dumont *et al.*, 2000; Frippiat *et al.*, 2001), proteotoxic stress (Balantinou *et al.*, 2009), or cell exposure to heavy metals (Katsiki *et al.*, 2004).

nCLU has been mostly correlated to a pro-death function that in certain cell types is mediated via nuclear localization and Ku70 binding after cell exposure to IR (Leskov *et al.*, 2003; Yang *et al.*, 2000) or due to its ability to dismantle the actin cytoskeleton (Moretti *et al.*, 2007) (see also chapter "Nuclear CLU (nCLU) and the Fate of the Cell"). On the other hand, there is some controversy about sCLU role during cell death execution mainly due to opposing reported functions (Trougakos and Gonos, 2002). More specifically, in certain cellular contexts sCLU may enhance apoptosis after cell exposure to stress- or apoptosis-inducing agents (e.g., photodynamic therapy, chemotherapy, and oxidants) (Chen *et al.*, 2004; Kalka *et al.*, 2000; Scaltriti *et al.*, 2004b; Trougakos *et al.*, 2005) and high amounts of CLU may become cytotoxic when accumulated intracellularly (Debure *et al.*, 2003; Trougakos *et al.*, 2005). In HeLa cells growing under constraint conditions, CLU inhibits the phosphatidylinositol-3′-kinase–Akt pathway through attenuation of insulin-like growth factor 1 (Jo *et al.*, 2008). Also, in CLU knockout ($CLU^{-/-}$) mice it was found that CLU may exacerbate neuronal damage in hypoxia-ischemia (Han *et al.*, 2001). Contrary to these findings, which may relate to cell overload with high amounts of sCLU, in the majority of reported studies transgene-mediated sCLU overexpression potently protected mammalian cells from a variety of apoptosis-inducing agents including various types of radiation, chemotherapeutic drugs, and histone deacetylase inhibitors (Criswell *et al.*, 2005; Hoeller *et al.*, 2005; Liu *et al.*, 2009; Miyake *et al.*, 2000b; Trougakos *et al.*, 2004, 2005; Xie *et al.*, 2005; Zellweger *et al.*, 2003; Zhang *et al.*, 2005). sCLU function during cell exposure to genuine oxidants was also cytoprotective. Finally, in a series of studies in $CLU^{-/-}$ mice, CLU was found to confer protection from autoimmune myocardial damage (McLaughlin *et al.*, 2000), permanent focal cerebral ischemia (Wehrli *et al.*, 2001), heat-shock-mediated apoptosis in the testis (Bailey *et al.*, 2002), and progressive age-related glomerulopathy (Rosenberg *et al.*, 2002). In support to the mainstream notion that favors a prosurvival role for sCLU (see also chapter "Cell Protective Functions of

Secretory CLU (sCLU)"), specific inhibition of its biosynthesis by either antisense oligonucleotides (ASOs) or small-interfering RNA (siRNA) resulted in significant cell sensitization to apoptosis induced by oxidative stress (Viard *et al.*, 1999), chemotherapeutic drugs (Hoeller *et al.*, 2005; Miyake *et al.*, 2000a; Trougakos *et al.*, 2004), IR (Criswell *et al.*, 2005), or TRAIL (Sallman *et al.*, 2007). Finally, it was recently found in prostate cancer cells that sCLU promoted survival by activating the prosurvival phosphatidylinositol-3-kinase/Akt pathway (Ammar and Closset, 2008).

Interestingly, an increasing number of reports indicate that even in the absence of external stress effective sCLU knock down reduces cell proliferation and increases the rates of endogenous spontaneous apoptosis in several normal or cancer mammalian cell types (Kang *et al.*, 2000; Moulson and Millis, 1999; Schlabach *et al.*, 2008; Sensibar *et al.*, 1995; Trougakos *et al.*, 2004, 2009b; Zwain and Amato, 2000). These findings open new paths in sCLU research as they clearly demonstrate the fundamental role of sCLU in suppressing endogenous cellular apoptotic signals and in the maintenance of cell homeostasis.

Recent efforts have provided some mechanistic models and shed light to sCLU implication in the regulation of the apoptotic machinery. More specifically, it was shown that CLU inhibits apoptosis by specifically interacting with conformation-altered activated Bax in the mitochondria (Zhang *et al.*, 2005). This study was corroborated by the recent finding that sCLU binds and thereby stabilizes the Ku70–Bax protein complex serving as a cytosol retention factor for Bax (Trougakos *et al.*, 2009b). These facts along with sCLU localization, both extracellularly and in various intracellular compartments, indicate that apart from its induction by oxidants in order to chaperone damaged proteins and reduce cell damage sCLU may also exert its chaperone function (intracellularly or extracellularly) even in the absence of stress having a vital housekeeping role in the maintenance of cellular proteome stability. This assumption is in line with previous reports as, apart from Ku70 and Bax binding in normally growing cells (Trougakos *et al.*, 2009b; Zhang *et al.*, 2005), sCLU also binds the TGF-β type I/II receptors (Reddy *et al.*, 1996), the cytosolic microtubule-destabilizing stathmin family protein SCLIP (Kang *et al.*, 2005) and phosphorylated IκBα (Devauchelle *et al.*, 2006).

B. Implication of Clusterin in States of Increased Organismal Oxidative Stress

During *in vivo* ageing, CLU expression levels were higher in the ventral and lateral prostate lobes of aged as compared to young rats (Lam *et al.*, 2008; Lau *et al.*, 2003; Omwancha *et al.*, 2009). Moreover, aging in rats impaired the serum lipid profile and increased lipid peroxidation, PON1

activities, and the content of both PON1 and sCLU in HDL which suggests an HDL subfraction redistribution to protect low-density lipoproteins more effectively from oxidation (Thomàs-Moyà *et al.*, 2006). In humans, sCLU was upregulated from gestation to adults (Wong *et al.*, 1994) and accumulated in the serum of elder subjects (Trougakos *et al.*, 2002b). Also it was found to be induced during ageing of the pituitary gland (Ishikawa *et al.*, 2006), glial cells (Patel *et al.*, 2004), lymphocytes (Trougakos *et al.*, 2006b), and adenohypophysis (Doğan Ekici *et al.*, 2008). Considering that ageing is among the main risk factors for many pathologies including vascular diseases, neurodegeneration, chronic inflammation, and cancer it is not surprising that CLU has been implicated in all these pathological conditions.

During vascular damage, sCLU was found to accumulate in the human serum of diabetes type II patients or during myocardial infraction (Trougakos *et al.*, 2002b). Also, sCLU accumulated in the artery wall during atherosclerosis where it was proposed to protect against the oxidative stress associated with the development of the disease (Mackness *et al.*, 1997). sCLU prevented fetal cardiac myoblast apoptosis induced by ethanol (Li *et al.*, 2007) and protected cardiomyocytes against ischemic cell death via a complement-independent pathway (Krijnen *et al.*, 2005). It was recently proposed that during atherogenesis the protective role of CLU may be exerted by binding to enzymatically modified low-density lipoprotein to reduce fatty acid-mediated cytotoxicity (Schwarz *et al.*, 2008).

Normal adult brain is a major site of CLU mRNA synthesis in several mammalian species and a significant increase in either mRNA or CLU protein expression has been observed during neurodegenerative conditions as well as during injury or chronic inflammation of the brain (Calero *et al.*, 2005). sCLU was found to associate with aggregated amyloid beta-peptide in senile and diffuse plaques of AD (Kida *et al.*, 1995), while in cortex and hippocampus extracts CLU concentration was about 40% higher in AD than in control individuals (Oda *et al.*, 1994). The role of CLU during neurodegeneration appeared controversial. Specifically, sCLU preincubation with Aβ before adding the mixture to PC12 cells enhanced the oxidation properties of Aβ and promoted its cytotoxicity (Oda *et al.*, 1995). On the other hand, sCLU was shown to prevent *in vitro* the Aβ peptide aggregation, polymerization, amyloid fibril formation, and neurotoxicity (Boggs *et al.*, 1996; Matsubara, *et al.*, 1996) as well as the spontaneous aggregation of a synthetic peptide homologous to human prion protein (McHattie and Edington, 1999). These discrepancies may be partially explained by the recent observation that the proamyloidogenic effects of CLU relate to the CLU:client protein ratio as when the substrate protein is present at a very large molar excess CLU coincorporates with it into insoluble aggregates, whereas when CLU is present in higher levels it potently inhibits amyloid formation and provides substantial cytoprotection (Yerbury *et al.*, 2007)

(see also chapter "The Chaperone Action of CLU and Its Putative Role in Quality Control of Extracellular Protein Folding"). Studies at *in vivo* models of PDAPP (homozygous for the APPV717F transgene)/CLU$^{-/-}$ mice showed a similar degree of Aβ deposition as compared with PDAPP/CLU$^{+/+}$ animals. However, the deposits were largely thioflavin negative in PDAPP/CLU$^{-/-}$ mice indicating that fewer amyloid deposits were formed (DeMattos *et al.*, 2002). Thus, according to this report, similarly to what previously proposed for Apolipoprotein E (Bales *et al.*, 1997), sCLU enhances Aβ toxicity by facilitating the conversion of soluble Aβ into amyloids. Intriguingly, it seems that Apolipoprotein E and CLU act cooperatively in the suppression of Aβ deposition as the double knockout PDAPP/ApoE$^{-/-}$/CLU$^{-/-}$ mice had an earlier onset and a significant increase in Aβ and amyloid deposition (DeMattos *et al.*, 2004).

Chronic inflammation results in increased oxidative stress and it thus represent an appropriate model to test CLU responsiveness to oxidant injury (see also chapter "CLU, Chronic Inflammation and Autoimmunity"). It was found that CLU expression levels correlated positively with the duration of juvenile dermatomyositis, a disease that introduces chronic inflammation in muscle (Chen *et al.*, 2008b), as well as that endotoxin, TNF-α, and IL-1 increased hepatic mRNA and serum protein levels of sCLU in Syrian hamsters (Hardardóttir *et al.*, 1994). Also CLU was upregulated following Venezuelan equine encephalitis virus infection (Sharma *et al.*, 2008) and found to exert a cytoprotective function in lung during leukocyte-induced injury (Heller *et al.*, 2003); in inflammatory exocrine pancreas (Savković *et al.*, 2007) and in glomerulonephritis (Rastaldi *et al.*, 2006). Finally, sCLU was induced during ventricular heart tissue damage but not in the hypertrophic tissue (Swertfeger *et al.*, 1996) while in acute sepsis and septic shock sCLU concentration in plasma was higher in septic patients as compared to controls (Kalenka, 2006). The cytoprotective role of sCLU is evident if we consider the observation that sCLU was highly upregulated in the serum of patients that survived sepsis and septic shock (~26.5- or 15-fold as compared to controls) whereas in nonsurvived patients sCLU upregulation was lower (5.9- to 3.1-fold) (Kalenka *et al.*, 2006).

In support to its proposed anti-inflammatory function, sCLU suppressed the pro-death activity of the inflammatory cytokine TNF-α in prostate cancer or mouse fibroblastic cells (Humphreys *et al.*, 1997; Sensibar *et al.*, 1995; Sintich *et al.*, 1999). Interestingly, in breast cancer cells treatment with TNF-α altered the biogenesis of endogenous CLU during apoptosis resulting in the appearance of a ~50 kDa uncleaved, nonglycosylated, disulfide-linked protein isoform of CLU that accumulates in the nucleus (O'Sullivan *et al.*, 2003).

In certain experimental models basal CLU gene expression or induction following cell exposure to stress depended on NF-κB activity (Li *et al.*, 2002; Saura *et al.*, 2003) (see also chapter "Regulation of CLU Gene Expression by Oncogenes, Epigenetic Regulation, and the Role of CLU in Tumorigenesis"). On the other hand, CLU itself mostly exerts a negative effect on NF-κB pathway activity indicating the existence of a negative feedback loop. Specifically, during proteinuria CLU inhibited NF-κB-dependent Bcl-X_L expression by stabilizating IkappaBalpha (Takase *et al.*, 2008). Similarly, in human neuroblastoma cells and murine embryonic fibroblasts CLU inhibited NF-κB by stabilizing IkappaBs (Santilli *et al.*, 2003), while in a model of mouse pancreatitis CLU reduced NF-κB activation and downstream expression of the NF-κB target genes TNF-α and MOB-1 (Savković *et al.*, 2007). In human synoviocytes CLU was found to suppress NF-κB-regulated cytokine production; it inhibited TNF-α-mediated NF-κB activation and interacted with phosphorylated IκB suppressing NF-κB nuclear translocation (Devauchelle *et al.*, 2006) (see also chapter "CLU, Chronic Inflammation and Autoimmunity").

Finally, CLU has been functionally implicated in all aspects of tumorigenesis including tumor formation and progression, metastasis, and chemoresistance acquisition (Shannan *et al.*, 2006; Trougakos and Gonos, 2002) (see also chapters "CLU and Prostate Cancer," "CLU and Breast Cancer," "CLU and Colon Cancer," and "CLU and Lung Cancer," this volume; and "Regulation of CLU Gene Expression by Oncogenes, Epigenetic Regulation, and the Role of CLU in Tumorigenesis," Vol. 105). Changes in CLU (mostly sCLU) expression have been documented in a broad variety of different human malignancies. According to studies in esophageal squamous cell carcinoma (Zhang *et al.*, 2003) and in low- and high-grade human prostate cancer (Scaltriti *et al.*, 2004a), tumor progression and malignancy correlate to decreased expression of CLU. Also high CLU expression levels were found to positively correlate with better survival in stage III serous ovarian adenocarcinomas (Partheen *et al.*, 2008) and cytoplasmic CLU expression is associated with longer survival in patients with non small cell lung cancer (Albert *et al.*, 2007). In other reported cases, however, CLU mRNA and protein upregulation was closely associated with disease progression. Specifically, higher CLU levels were detected in kidney (Parczyk *et al.*, 1994) and prostate carcinomas (Steinberg *et al.*, 1997), in anaplastic large-cell lymphomas (Wellmann *et al.*, 2000), in ovarian cancer (Hough *et al.*, 2001), in human hepatocellular carcinoma (Kang *et al.*, 2004), in primary melanoma and melanoma metastases (Hoeller *et al.*, 2005), in leukemic non-Hodgkin's lymphomas (Chow *et al.*, 2006), in endometrioid and cervical carcinoma (Abdul-Rahman *et al.*, 2007; Ahn *et al.*, 2008), in pancreatic endocrine tumors (Mourra *et al.*, 2007), and during seminal vesicle carcinogenesis progression (Ouyang *et al.*, 2001). sCLU has been characterized as a

sensitive biomarker of murine and human intestinal tumors (Chen *et al.*, 2003), anaplastic large-cell lymphomas (Saffer *et al.*, 2002), colon (Chen *et al.*, 2008a; Pucci *et al.*, 2004, 2009) and breast carcinomas (Redondo *et al.*, 2002). Moreover, high CLU expression correlated to the aggressiveness of breast carcinomas (Redondo *et al.*, 2002) and with a poor outcome in stage II colorectal cancers (Kevans *et al.*, 2009). In human lung adenocarcinoma cell lines CLU positively regulated EMT and aggressive behavior through modulating ERK signaling and Slug expression (Chou *et al.*, 2009). In support, sCLU has been found to be a potentially new prognostic and predictive marker for tumor metastasis in hepatocellular (Lau *et al.*, 2006) and colon carcinomas (Pucci *et al.*, 2004, 2009). Protemic analysis in colorectal cancer patients revealed the presence of several CLU isoforms that were distinct in their isoelectric point and glycozylation pattern; some of these were induced in tumor samples and thus could have a potential value as colorectal tumor markers (Rodríguez-Piñeiro *et al.*, 2006). Finally, sCLU appeared to be associated with resistance to chemotherapy treatments *in vivo* (Zellweger *et al.*, 2003) and it is significantly overexpressed in multidrug resistant osteosarcoma cell lines (Lourda *et al.*, 2007) and in docetaxel-resistant prostate tumor cells (Patterson *et al.*, 2006) (see also chapter "CLU and Chemoresistance," Vol. 105). CLU was also found to interact with Paclitaxel and conferred Paclitaxel resistance in ovarian cancer (Park *et al.*, 2008). A direct proof of CLU involvement in tumor cells resistance to chemotherapy relates to the fact that direct inhibition of CLU biosynthesis by siRNA (Lourda *et al.*, 2007) or indirect by resveratrol (Patterson *et al.*, 2006; Sallman *et al.*, 2007) resensitized the drug-resistant cancer cells to the chemotherapeutic drugs used.

Intriguingly, although sCLU overexpression in Myc-transduced colonocytes decreased cell accumulation (Thomas Tikhonenko *et al.*, 2004), it promoted c-Myc-mediated transformation in Rat-1 cells *in vitro* and tumor progression *in vivo* into nude mice (Zhang *et al.*, 2005). In a recent study where the role of CLU during tumor development was investigated in mouse models of neuroblastoma, development of the disease and metastasis showed a negative correlation with CLU expression levels suggesting that, in this particular type of cancer, CLU is a tumor and metastasis suppressor gene (Chayka *et al.*, 2009). However, the picture gets more complicated if we also consider another recent study showing that loss of Nkx3.1, a transcriptional regulator and tumor suppressor gene in prostate cancer that is downregulated during early stages of prostate tumorigenesis, resulted in CLU aberrant overexpression in Nkx3.1-driven tumor initiation (Song *et al.*, 2009). This finding was investigated in independent loss-of-PTEN and c-myc overexpression prostate adenocarcinoma mouse models where Nkx3.1 loss is an early event or late event, respectively. CLU was found to be highly expressed in prostatic hyperplasia and intraepithelial neoplasia lesions that also lack

Nkx3.1 in the PTEN-deficient prostate, but not in similar lesions in the c-myc transgenic model. Meta-analysis of multiple prostate cancer gene expression data sets, including those from loss-of-Nkx3.1, loss-of-PTEN, c-myc overexpression, and constitutively active AKT prostate cancer models, further confirmed that genes associated with the loss-of-Nkx3.1 signature (like CLU) integrate with PTEN–AKT signaling pathways, but do not overlap with molecular changes associated with the c-myc signaling pathway. In human prostate tissue samples, loss of NKX3.1 expression and CLU overexpression are colocalized at sites of prostatic inflammatory atrophy, a possible very early stage of human prostate tumorigenesis (Song *et al.*, 2009). Thus, in this model CLU seems to actively participate in the early phases of prostate cancer. Overall, the link between CLU expression and cancer progression seems to be complicated and may depend on the type of cancer, the oncogenic pathways activated, the endogenous CLU expression levels in the cell or animal model used, and on the tissue or cell-type studied.

C. What Can We Learn from the Functional Similarities Between Clusterin and Its Distal Homologues, the Small-Heat Shock Proteins?

HSPs induction upon exposure to environmental insults constitutes one of the most evolutionarily conserved stress responses known to the living world. Molecular chaperones are a large family of ubiquitous proteins that appeared early in evolution to form a defensive system in cells by sequestering damaged proteins and preventing their aggregation (Soti *et al.*, 2005; Sun and McRae, 2005). HSPs comprise a group of related proteins classified into six major families according to their molecular weight (Martindale and Holbrook, 2002); from these, as mentioned, sCLU shares functional similarities with sHSPs (Carver *et al.*, 2003). Only few members of the sHSPs family [e.g., the mammalian HSP27 (HSPB1) and aB-crystallin (HSPB5) proteins] display the features of a genuine HSP and are induced in response to stress (Arrigo *et al.*, 2007).

As already described for CLU, the induction of HSPs in response to stress is largely mediated through transcriptional activation via HSF1 (Pirkkala *et al.*, 2001). The common activating signal is likely to be oxidative stress and the consequent oxidative damage to proteins that alters their folding or introduce oxidative-inflammation conditions (Arrigo *et al.*, 2007). In most mammals, sHSPs are constitutively expressed in tissues with high rates of oxidative metabolism, including, the heart, type I and type IIa skeletal muscle fibers, brain, and oxidative regions of the kidney (Arrigo, 2001). The similar expression pattern of CLU in these tissues offers an additional

line of defense in the organism to combat oxidative stress. However, the accumulation of a series of different regulatory elements in the CLU gene promoter makes CLU gene more sensitive than sHSPs in the various environmental insults.

sHSPs are molecular chaperones and function to protect proteome against denaturation or oxidative inactivation (Arrigo, 2001; Jolly and Morimoto, 2000). As in the case of CLU, insults that may activate sHSPs include either direct oxidative damage (e.g., exposure to hydrogen peroxide or hypoxia reperfusion injury) or a variety of other stresses in which generation of oxygen-free radicals is indirect (e.g., chemotherapeutic drugs, heat stress, cytokines, etc.) (Graf and Jakob, 2002; Martindale and Holbrook, 2002). Elevated sHSPs expression improves cell survival by reducing the oxidative damage to DNA, proteins, and lipids (Park *et al.*, 1998; Su *et al.*, 1999; Yamamoto *et al.*, 2000) and by decreasing the intracellular levels of free radicals and their derivatives as they modulate metabolism of glutathione to maintain it in a reduced state (Baek *et al.*, 2000; Preville *et al.*, 1999). Reportedly, sHSPs preserve proteasome function (Ding and Keller, 2001), involve in cytoskeleton stabilization (Singh *et al.*, 2007), and suppress apoptotic signaling pathways downstream of mitochondria at the level of cytochrome *c* and apoptosome (Bruey *et al.*, 2000; Paul *et al.*, 2002). Also sHSPs interact with caspases (Kamradt *et al.*, 2001) and suppress Bax, Bcl-X_S, and p53 polypeptides translocation to the mitochondria (Liu *et al.*, 2007; Mao *et al.*, 2004). As has been reported for sCLU, sHSPs overexpression have been shown to enhance survival of cells and prevent apoptosis during a wide variety of stress conditions (Creagh *et al.*, 2000; Jolly and Morimoto, 2000). Apart from being expressed in stressed cells, some sHSPs show a basal level of constitutive expression and act as in-house chaperone toward several fundamental cellular processes, such as cytoskeletal architecture, mutations masking, protein intracellular transport, intracellular redox homeostasis, and translation regulation (Arrigo *et al.*, 2007). The fact that sCLU shows a constitutive pattern of expression in many cell types indicates a similar profile of housekeeping functions as was recently proposed (Trougakos *et al.*, 2009b).

Additional functional similarities between sCLU and sHSPs relate to their reported implication in various pathological conditions including *in vitro* senescence and ageing (Soti and Csermely, 2003), carcinogenesis, metastasis, and drug resistance (Aldrian *et al.*, 2002; Aoyama *et al.*, 1993; Chelouche-Lev *et al.*, 2004; Elpek *et al.*, 2003; Ungar *et al.*, 1994). Also, sHSPs have been functionally implicated in heart attack and stroke (Hollander *et al.*, 2004; Ray *et al.*, 2001) where HSP27 transgenic mice are strongly protected against myocardial infarction and cerebral ischemia (Efthymiou *et al.*, 2004); in neurodegenation (Renkawek *et al.*, 1994; Shinohara *et al.*, 1993; Yoo *et al.*, 2001); as well as in inflammatory diseases (Alford *et al.*, 2007).

Nonetheless, similarly to sCLU, sHSPs role in these diseases is dual as they may either exacerbate toxicity or function cytoprotectively (Calderwood and Ciocca, 2008).

A supposed main difference between sCLU and sHSPs seemed to be their differential localization. sHSPs are intracellular chaperones localized mainly in the cytosol and nucleus (Arrigo, 2007), whereas sCLU because of the ER-targeting hydrophobic signal peptide were expected to reside in the membranous compartments of the cell (e.g., ER, Golgi, secretory vesicles, and probably plasma membrane) as well as in the extracellular milieu (Calero *et al.*, 2005; Shannan *et al.*, 2006; Trougakos and Gonos, 2002). Most intriguingly, however, recent reports indicate that either under stress or even at normal conditions sCLU can escape, by a currently unknown mechanism, the membranous network of the cell and localize in the nucleus, cytosol, or mitochondria (Caccamo *et al.*, 2006; Kang *et al.*, 2005; O'Sullivan *et al.*, 2003; Trougakos *et al.*, 2009b; Zhang *et al.*, 2005). Although further studies are needed to verify these findings, they raise the possibility that sCLU may represent the only chaperone that confers proteome stability both extra- and intracellularly.

VI. CONCLUDING REMARKS—PERSPECTIVES

From a first sight the function of CLU seems elusive the main cause being the opposing functions proposed in an array of various cell types and tissues. CLU seems to be an "*all in one*" super protein as its full-length version has signal peptides for both secretion and nuclear localization; it can bind a wide range of totally unrelated molecules and carries a wide array of different posttranslational modifications. On top of this, CLU gene promoter contains a collection of many distinct regulatory elements that have the potential to respond to even minute environmental changes or alterations in cellular homeostasis. Besides these unique properties, our protein homology searching in the existing databases revealed the existence of CLU-like protein sequences only in vertebrates. Also, a comparison of the CLU gene promoter DNA or the conventional full-length sCLU protein sequences among mammalian species showed a very high degree of conservation. These facts, along with the wide tissue distribution of CLU (Aronow *et al.*, 1993), its implication in normal processes like development and differentiation (Garden *et al.*, 1991; Itahana *et al.*, 2007; O'Bryan *et al.*, 1993; Trougakos *et al.*, 2006a) and the absence of functional CLU gene polymorphisms in humans (Trougakos *et al.*, 2006b; Tycko *et al.*, 1996) clearly indicate that the protein has evolved in vertebrates to accomplish a function of fundamental biological importance.

After a period of debate the emerging consensus is that sCLU is mainly prosurvival, through its chaperone activity, whereas nCLU, when expressed, promotes cell death. Regarding sCLU, the idea that it cannot be "harmful *per se*" for the cells is mostly apparent by the facts that as mentioned it has a ubiquitous constitutive expression in most adult human tissues and it associates with cells surviving apoptosis during development and differentiation. The future discovery of more protein isoforms (e.g., those supposed to be primates specific; see also chapter "Regulation of CLU Gene Expression by Oncogenes, Epigenetic Regulation, and the Role of CLU in Tumorigenesis," Vol. 105) may add some complexity to that picture but we foresee that it will not dramatically alter our current ideas about CLU protein(s) sequence-derived function because all these isoforms are expected to differ in less than 10% of their N-terminus sequence. This difference although may impact on the localization pattern of the produced polypeptide(s) should not significantly affect the primary functional properties of the produced protein.

It is anticipated that the property of sCLU to chaperone unfolded damaged proteins most probably denotes its primary function. Based on the up to date observations and the CLU gene structure it is reasonable to argue that CLU is a sensitive biosensor of environmental insults and more particularly oxidative stress that is the driving force of most, if not all, age-related diseases (including cancer) where CLU has been functionally implicated. In these pathological conditions, the main mission of CLU would be to cease the deleterious effects of oxidative stress. If this hypothesis is true, then CLU gene expression and protein upregulation during ageing should reflect the general "oxidative status" of the subject rather than its chronological age. Indeed, although CLU gene expression levels in lymphocyte samples are higher in old donors as compared to their young counterparts, in similar samples from centenarians [a model of successful ageing characterized by low ROS load (Franceschi and Bonafe, 2003)], CLU gene expression levels were significantly lower than those found in the old donors (Trougakos *et al.*, 2006b).

On the basis of its chaperone activity and the supposed "beneficial" role of sCLU at the cellular level it is anticipated that there would be a selective (Darwinian) pressure for higher sCLU expression levels in both normal and pathological cells. However, the outcome at the organismal level may vary dramatically when we consider the added layers of complexity and the fact that what is "beneficial" at the cellular level may eventually become suicidal for the host; this argument mostly applies for cancer cells. Thus, sCLU implication in biological systems should be considered in a holistic view rather than focusing in a particular cellular state or disease (Fig. 2).

sCLU is neither a typical oncogene nor a typical tumor suppressor as according to current knowledge it cannot be classified as a classical

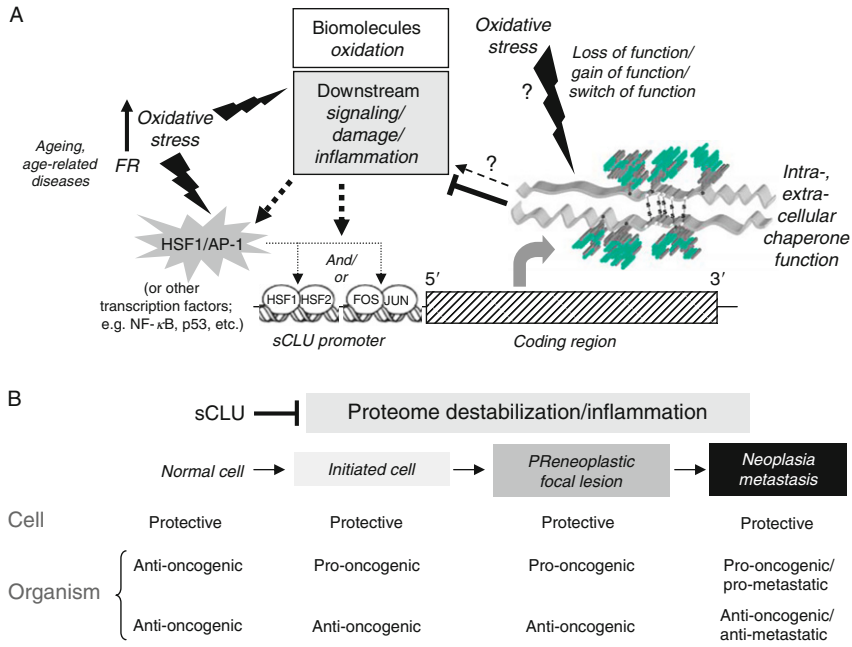

Fig. 2 Proposed CLU gene regulation and function as a response to altered oxygen-free radicals concentration. (A) As CLU promoter contains binding sites for HSF and AP-1 transcription factors complexes it is very sensitive to oxidative stress. CLU gene can also be regulated by the huge repertoire of molecules (e.g., cytokines, growth factors, etc.) produced as signaling (by)-products of oxidative stress and/or the accompanying inflammation. sCLU mostly suppress the toxic effects of oxidation (⊤), although it cannot be excluded that in certain cellular contexts, it may have a protoxic effect (↑). sCLU itself may also be subject to oxidative stress-induced modifications that may affect its function. (B) It is anticipated that at the cellular level sCLU mostly functions cytoprotectively. However, at the organismal level this property may suppress or promote tumor formation depending on various parameters such as the cell type or tissue characteristics; the endogenous levels of expression and isoforms (e.g., sCLU, nCLU) involved; the oncogenic pathways activated and the tissue microenvironment. FR, free radicals.

initiator or effector of cell cycle or death regulation (Trougakos *et al.*, 2009a). Its role seems to be more complicated as in early stages of cancer sCLU may indirectly act as a tumor suppressor due to its ability to suppress the accumulation of damaged proteins. However, during the initiation of neoplasia or at established tumors sCLU function may become oncogenic because of its ability to counteract apoptosis, either indirectly by actively involved in damage repair (Carver *et al.*, 2003) or directly by interfering with Bax pro-death activity (Trougakos *et al.*, 2009b; Zhang *et al.*, 2005), allowing thus cancer cells to acquire one of the main cancer hallmarks,

namely resistance to apoptosis. Experimental evidence to support the "two-faced" character of CLU during tumorigenesis come from the observation that sCLU might act in a biphasic fashion during skin carcinogenesis as a tumor attenuator in early carcinogenesis and as an enhancer of the malignant phenotype in late carcinogenesis (Thomas-Tikhonenko *et al.*, 2004). Even in those cases where at the early phases of carcinogenesis the tumor microenvironment (e.g., aberrant oncogene activation) suppresses sCLU expression (Lund *et al.*, 2006) the selective pressure for CLU gene reactivation as the tumor evolves may eventually bypass the suppressing mechanisms allowing cells to reexpress sCLU. This theoretical model may explain recent reports showing that few nests of cancer cells, in an otherwise sCLU negative tumor, express high levels of the protein (Scaltriti *et al.*, 2004a) most probably due to *de novo* synthesis (Andersen *et al.*, 2007). It would be critical to address the question whether these tumor cells have a more aggressive phenotype as compared to the surrounding tissue. Apparently, the outcome of CLU contribution to tumor initiation and progression may also be affected by several other parameters including the cell type or tissue characteristics (i.e., the genetic background), the CLU endogenous expression levels and isoforms(s) implicated, the oncogenic stimuli and pathways activated, the tumor microenvironment and finally, the site of action.

In conclusion, the observations discussed herein, suggest that sCLU could be a valuable prognostic biomarker of increased organismal stress and disease progression. Also, sCLU, or other CLU protein isoforms, represent promising targets for the development of future therapeutic strategies against pathologies as diverse as cancers, inflammation, neurodegenerative diseases, or cataracts. Nevertheless, although it is anticipated that sCLU activation would be beneficial for the organism if we consider pathologies like atherosclerosis, degenerative diseases, or inflammation, the decision whether sCLU should be activated or inhibited in a certain tumor should take several parameters into consideration.

For instance, inhibition of NF-κB activation is now widely recognized as a valid drug-target strategy to combat inflammatory disease or cancer (Sarkar *et al.*, 2008). As CLU seems to be an NF-κB inhibitor its activation may has certain theoretical advantages in cancer therapy. sCLU activation may also provide therapeutic benefits in those tumors where sCLU exerts a tumor suppressing action (Chayka *et al.*, 2009). In addition, nCLU activation by natural compounds used either solely or in combination with chemotherapeutic drugs, may represent another therapeutic endeavor.

The alternative option of sCLU gene expression inhibition in tumor cells is currently exploited by OGX-011, a 2′-methoxyethyl-modified phosphorothioate ASO that is complementary to CLU mRNA (Chi *et al.*, 2008; Miyake *et al.*, 2000c). OGX-011 is in clinical trials for several types of

cancer. Phase I trials showed that OGX-011 is well tolerated in patients, reduces CLU expression in prostate cancer, and enhances the apoptotic responses (Chi *et al.*, 2005). According to a recent announcement of the final results from phase II clinical trials in patients receiving chemotherapy treatment for castrate-resistant prostate cancer (*http://www.oncogenex.com/*) treatment with an OGX-011/docetaxel combination was independently associated with improved overall patient survival in a preplanned multivariate analysis. Work in progress aims to finalize plans for two, phase III studies.

These latest exciting findings urge prompt investigation of various topics in CLU biology including: (a) its role in mitochondria and the additional activities (besides its chaperone function) needed to fight against apoptotic cell death; (b) its implication in the regulation of cell signaling pathways; (c) the molecular regulation, function, and localization of the various CLU protein isoforms in distinct cell types and tissues; (d) the isolation of novel extra- or intracellular binding partners; (e) its oxidation status during ageing or at the various age-related diseases and finally; (f) the detailed mapping and responsiveness to oxidative stress of the promoter(s) that regulate the various CLU isoforms. Last, but not least, the development of novel CLU overexpressing animal models along with the elucidation of CLU's crystal structure will provide precious information in our attempts to understand the function of this fascinating protein.

In any case, we should always bear in mind that in view of the integrative nature of living systems, the phenotypic outcome of stress-induced, homeostasis assuring proteins (like sCLU) in multifactorial diseases like cancer may be affected by several parameters resulting to either an antioncogenic or to a prooncogenic effect.

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REFERENCES

Abdul-Rahman, P. S., Lim, B. K., and Hashim, O. H. (2007). Expression of high-abundance proteins in sera of patients with endometrial and cervical cancers: Analysis using 2-DE with silver staining and lectin detection 7methods. *Electrophoresis* **28**, 1989–1996.

Ahn, H. J., Bae, J., Lee, S., Ko, J. E., Yoon, S., Kim, S. J., and Sakuragi, N. (2008). Differential expression of clusterin according to histological type of endometrial carcinoma. *Gynecol. Oncol.* **110,** 222–229.

Albert, J. M., Gonzalez, A., Massion, P. P., Chen, H., Olson, S. J., Shyr, Y., Diaz, R., Lambright, E. S., Sandler, A., Carbone, D. P., Putnam, J. B., Jr., Johnson, D. H., *et al.* (2007). Cytoplasmic clusterin expression is associated with longer survival in patients with resected non small cell lung cancer. *Cancer Epidemiol. Biomarkers Prev.* **16,** 1845–1851.

Aldrian, S., Trautinger, F., Frohlich, I., Berger, W., Micksche, M., and Kindas-Mugge, I. (2002). Overexpression of Hsp27 affects the metastatic phenotype of human melanoma cells *in vitro*. *Cell Stress Chaperones* **7,** 177–185.

Alexandrova, M., Bochev, P., Markova, V., Bechev, B., Popova, M., Danovska, M., and Simeonova, V. (2004). Dynamics of free radical processes in acute ischemic stroke: Influence on neurological status and outcome. *J. Clin. Neurosci.* **11,** 501–506.

Alexopoulos, E. C., Cominos, X., Trougakos, I. P., Lourda, M., Gonos, E. S., and Makropoulos, V. (2008). Biological monitoring of hexavalent chromium and serum levels of the senescence biomarker apolipoprotein j/clusterin in welders. *Bioinorg. Chem. Appl.* **2008,** 420578.

Alford, K. A., Glennie, S., Turrell, B. R., Rawlinson, L., Saklatvala, J., and Dean, J. L. (2007). Heat shock protein 27 functions in inflammatory gene expression and transforming growth factor-beta-activated kinase-1 (TAK1)-mediated signaling. *J. Biol. Chem.* **282,** 6232–6241.

Ammar, H., and Closset, J. L. (2008). Clusterin activates survival through the phosphatidylinositol 3-kinase/Akt pathway. *J. Biol. Chem.* **283,** 12851–12861.

Andersen, C. L., Schepeler, T., Thorsen, K., Birkenkamp-Demtröder, K., Mansilla, F., Aaltonen, L. A., Laurberg, S., and Ørntoft, T. F. (2007). Clusterin expression in normal mucosa and colorectal cancer. *Mol. Cell. Proteomics* **6,** 1039–1048.

Aoyama, A., Steiger, R. H., Frohli, E., Schafer, R., von Deimling, A., Wiestler, O. D., and Klemenz, R. (1993). Expression of aB-crystallin in human brain tumors. *Int. J. Cancer* **55,** 760–764.

Aronow, B. J., Lund, S. D., Brown, T. L., Harmony, J. A. K., and Witte, D. P. (1993). Apolipoprotein J expression at fluid-tissue interfaces: Potential role in barrier cytoprotection. *Proc. Natl. Acad. Sci. USA* **90,** 725–729.

Arrigo, A. P. (2001). Hsp27: Novel regulator of intracellular redox state. *IUBMB Life* **52,** 303–307.

Arrigo, A. P., Simon, S., Gibert, B., Kretz-Remy, C., Nivon, M., Czekalla, A., Guillet, D., Moulin, M., Diaz-Latoud, C., and Vicart, P. (2007). Hsp27 (HspB1) and alphaB-crystallin (HspB5) as therapeutic targets. *FEBS Lett.* **581,** 3665–3674.

Baek, S. H., Min, J. N., Park, E. M., Han, M. Y., Lee, Y. S., Lee, Y. J., and Park, Y. M. (2000). Role of small heat shock protein HSP25 in radioresistance and glutathione-redox cycle. *J. Cell. Physiol.* **183,** 100–107.

Bailey, R. W., Aronow, B., Harmony, J. A., and Griswold, M. D. (2002). Heat shock-initiated apoptosis is accelerated and removal of damaged cells is delayed in the testis of clusterin/ApoJ knock-out mice. *Biol. Reprod.* **66,** 1042–1053.

Balantinou, E., Trougakos, I. P., Chondrogianni, N., Margaritis, L. H., and Gonos, E. S. (2009). Transcriptional and post-translational regulation of clusterin by the two main cellular proteolytic pathways. *Free Radic. Biol. Med.* **46,** 1267–1274.

Bales, K. R., Verina, T., Dodel, R. C., Du, Y., Altstiel, L., Bender, M., Hyslop, P., Johnstone, E. M., Little, S. P., Cummins, D. J., Piccardo, P., Ghetti, B., *et al.* (1997). Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat. Genet.* **17,** 263–264.

Batchioglu, K., Mehmet, N., Ozturk, I. C., Yilmaz, M., Aydogdu, N., Erguvan, R., Uyumlu, B., Genc, M., and Karagozler, A. A. (2006). Lipid peroxidation and antioxidant status in stomach cancer. *Cancer Invest.* **24,** 18–21.

Bauerova, K., and Bezek, A. (1999). Role of reactive oxygen and nitrogen species in etiopathogenesis of rheumatoid arthritis. *Gen. Physiol. Biophys.* **18,** 15–20.

Beckman, K. B., and Ames, B. N. (1998). The free radical theory of aging matures. *Physiol. Rev.* **78,** 547–581.

Bellomo, G., and Mirabelli, F. (1992). Oxidative stress and cytoskeletal alterations. *Ann. N. Y. Acad. Sci.* **663,** 97–109.

Bettuzzi, S., Scorcioni, F., Astancolle, S., Davalli, P., Scaltriti, M., and Corti, A. (2002). Clusterin (SGP-2) transient overexpression decreases proliferation rate of SV40-immortalized human prostate epithelial cells by slowing down cell cycle progression. *Oncogene* **21,** 4328–4334.

Boggs, L. N., Fuson, K. S., Baez, M., Churgay, L., McClure, D., Becker, G., and May, P. C. (1996). Clusterin (Apo J) protects against *in vitro* amyloid-beta(1–40) neurotoxicity. *J. Neurochem.* **67,** 1324–1327.

Bruey, J. M., Ducasse, C., Bonniaud, P., Ravagnan, L., Susin, S. A., Diaz-Latoud, C., Gurbuxani, S., Arrigo, A. P., Kroemer, G., Solary, E., and Garrido, C. (2000). Hsp27 negatively regulates cell death by interacting with cytochrome *c*. *Nat. Cell Biol.* **2,** 645–652.

Caccamo, A. E., Desenzani, S., Belloni, L., Borghetti, A. F., and Bettuzzi, S. (2006). Nuclear clusterin accumulation during heat shock response: Implications for cell survival and thermotolerance induction in immortalized and prostate cancer cells. *J. Cell. Physiol.* **207,** 208–219.

Calderwood, S. K., and Ciocca, D. R. (2008). Heat shock proteins: Stress proteins with Janus-like properties in cancer. *Int. J. Hyperthermia* **24,** 31–39.

Calero, M., Rostagno, A., Frangione, B., and Ghiso, J. (2005). Clusterin and Alzheimer's disease. *Subcell. Biochem.* **38,** 273–298.

Campisi, J. (2005). Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. *Cell* **120,** 513–522.

Carmeliet, P. (2000). Mechanisms of angiogenesis and arteriogenesis. *Nat. Med.* **6,** 389–395.

Carnevali, S., Luppi, F., D'Arca, D., Caporali, A., Ruggieri, M. P., Vettori, M. V., Caglieri, A., Astancolle, S., Panico, F., Davalli, P., Mutti, A., Fabbri, L. M., *et al.* (2006). Clusterin decreases oxidative stress in lung fibroblasts exposed to cigarette Smoke. *Am. J. Respir. Crit. Care Med.* **174,** 393–399.

Carrard, G., Bulteau, A. L., Petropoulos, I., and Friguet, B. (2002). Impairment of proteasome structure and function in aging. *Int. J. Biochem. Cell. Biol.* **34,** 1461–1474.

Carver, J. A., Rekas, A., Thorn, D. C., and Wilson, M. R. (2003). Small heat-shock proteins and clusterin: Intra- and extracellular molecular chaperones with a common mechanism of action and function? *IUBMB Life* **55,** 661–668.

Cavalli, L. R., and Liang, B. D. (1998). Mutagenesis, tumorigenicity, and apoptosis: Are the mitochondria involved? *Mutat. Res.* **398,** 19–26.

Chaiswing, L., Bourdeau-Heller, J. M., Zong, W., and Oberley, T. D. (2007). Characterization of redox state of two human prostate carcinoma cell lines with different degrees of aggressiveness. *Free Radic. Biol. Med.* **43,** 202–215.

Chayka, O., Corvetta, D., Dews, M., Caccamo, A. E., Piotrowska, I., Santilli, G., Gibson, S., Sebire, N. J., Himoudi, N., Hogarty, M. D., Anderson, J., Bettuzzi, S., *et al.* (2009). Clusterin, a haploinsufficient tumor suppressor gene in neuroblastomas. *J. Natl Cancer Inst.* **101,** 663–677.

Chelouche-Lev, D., Kluger, H. M., Berger, A. J., Rimm, D. L., and Price, J. E. (2004). aB-crystallin as a marker of lymph node involvement in breast carcinoma. *Cancer* **100,** 2543–2548.

Chen, F., Castranova, V., and Shi, X. (2001). New insights into the role of nuclear factor-B in cell growth regulation. *Am. J. Pathol.* **159,** 387–397.

Chen, X., Halberg, R. B., Ehrhardt, W. M., Torrealba, J., and Dover, W. F. (2003). Clusterin as a biomarker in murine and human intestinal neoplasia. *Proc. Natl. Acad. Sci. USA* **100,** 9530–9535.

Chen, T., Turner, J., McCarthy, S., Scaltriti, M., Bettuzzi, S., and Yeatman, T. J. (2004). Clusterin-mediated apoptosis is regulated by adenomatous polyposis coli and is p21 dependent but p53 independent. *Cancer Res.* **64,** 7412–7419.

Chen, X., Ehrhardt, W. M., Halberg, R. B., Aronow, B. J., and Dove, W. F. (2008a). Cellular expression patterns of genes upregulated in murine and human colonic neoplasms. *J. Histochem. Cytochem.* **56,** 433–441.

Chen, Y. W., Shi, R., Geraci, N., Shrestha, S., Gordish-Dressman, H., and Pachman, L. M. (2008b). Duration of chronic inflammation alters gene expression in muscle from untreated girls with juvenile dermatomyositis. *BMC Immunol.* **9,** 43.

Chen, J., Velalar, C. N., and Ruan, R. (2008c). Identifying the changes in gene profiles regulating the amelioration of age-related oxidative damages in kidney tissue of rats by the intervention of adult-onset calorie restriction. *Rejuvenation Res.* **11,** 757–763.

Chi, K. N., Eisenhauer, E., Fazli, L., Jones, E. C., Goldenberg, S. L., Powers, J., Tu, D., and Gleave, M. E. (2005). A phase I pharmacokinetic and pharmacodynamic study of OGX-011, a 2′-methoxyethyl antisense oligonucleotide to clusterin, in patients with localized prostate cancer. *J. Natl Cancer Inst.* **97,** 1287–1296.

Chi, K. N., Zoubeidi, A., and Gleave, M. E. (2008). Custirsen (OGX-011): A second-generation antisense inhibitor of clusterin for the treatment of cancer. *Expert Opin. Investig. Drugs* **17,** 1955–1962.

Chinenov, Y., and Kerppola, T. K. (2001). Close encounters of many kinds: Fos–Jun interactions that mediate transcription regulatory specificity. *Oncogene* **20,** 2438–2452.

Chondrogianni, N., and Gonos, E. S. (2005). Proteasome dysfunction in mammalian aging: Steps and factors involved. *Exp. Gerontol.* **40,** 931–938.

Chou, T. Y., Chen, W. C., Lee, A. C., Hung, S. M., Shih, N. Y., and Chen, M. Y. (2009). Clusterin silencing in human lung adenocarcinoma cells induces a mesenchymal-to-epithelial transition through modulating the ERK/Slug pathway. *Cell Signal.* **21,** 704–711.

Chow, K. U., Nowak, D., Kim, S. Z., Schneider, B., Komor, M., Boehrer, S., Mitrou, P. S., Hoelzer, D., Weidmann, E., and Hofmann, W. K. (2006). *In vivo* drugresponse in patients with leukemic non-Hodgkin's lymphomas is associated with *in vitro* chemosensitivity and gene expression profiling. *Pharmacol. Res.* **53,** 49–61.

Collado, M., and Serrano, M. (2006). Innovation: The power and the promise of oncogene-induced senescence markers. *Nat. Rev. Cancer* **6,** 472–476.

Creagh, E. M., Sheehan, D., and Cotter, T. G. (2000). Heat shock proteins—Modulators of apoptosis in tumour cells. *Leukemia* **14,** 1161–1173.

Criswell, T., Beman, M., Araki, S., Leskov, K., Cataldo, E., Mayo, L. D., and Boothman, D. A. (2005). Delayed activation of insulin-like growth factor-1 receptor/Src/MAPK/Egr-1 signaling regulates clusterin expression, a pro-survival factor. *J. Biol. Chem.* **280,** 14212–14221.

Debacq-Chainiaux, F., Borlon, C., Pascal, T., Royer, V., Eliaers, F., Ninane, N., Carrard, G., Friguet, B., de Longueville, F., Boffe, S., Remacle, J., and Toussaint, O. (2005). Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway. *J. Cell Sci.* **118,** 743–758.

Debure, L., Vayssiere, J.L, Rincheval, V., Loison, F., Le Drean, Y., and Michel, D. (2003). Intracellular clusterin causes juxtanuclear aggregate formation and mitochondrial alteration. *J. Cell Sci.* **116,** 3109–3121.

DeMattos, R. B., O'dell, M. A., Parsadanian, M., Taylor, J. W., Harmony, J. A. K., Bales, K. R., Paul, S. M., Aronow, B. J., and Holtzman, D. M. (2002). Clusterin promotes amyloid plaque formation and is critical for neuritic toxicity in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **99,** 10843–10848.

DeMattos, R. B., Cirrito, J. R., Parsadanian, M., May, P. C., O'Dell, M. A., Taylor, J. W., Harmony, J. A., Aronowv, B. J., Bales, K. R., Paul, S. M., and Holtzman, D. M. (2004). ApoE and clusterin cooperatively suppress Abeta levels and deposition. Evidence that ApoE regulates extracellular Abeta metabolism *In Vivo*. *Neuron* **41,** 193–202.

Demple, B., and Harrison, L. (1994). Repair of oxidative damage to DNA. *Annu. Rev. Biochem.* **63,** 915–948.

de Silva, H. V., Stuart, W. D., Duvic, C. R., Wetterau, J. R., Ray, M. J., Ferguson, D. G., Albers, H. W., Smith, W. R., and Harmony, J. A. (1990). A 70-kDa apolipoprotein designated ApoJ is a marker for subclasses of human plasma high density lipoproteins. *J. Biol. Chem.* **265,** 13240–13247.

Devauchelle, V., Essabbani, A., De Pinieux, G., Germain, S., Tourneur, L., Mistou, S., Margottin-Goguet, F., Anract, P., Migaud, H., Le Nen, D., Lequerré, T., Saraux, A., *et al.* (2006). Characterization and functional consequences of underexpression of clusterin in rheumatoid arthritis. *J. Immunol.* **177,** 6471–6479.

Devi, G. S., Prasad, M. H., Saraswathi, I., Raghu, D., Rao, D. N., and Reddy, P. P. (2000). Free radicals antioxidant enzymes and lipid peroxidation in different types of leukemia. *Clin. Chim. Acta* **293,** 53–62.

de Visser, K. E., Korets, L. V., and Coussens, L. M. (2005). *De novo* carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* **7,** 411–423.

Ding, Q., and Keller, J. N. (2001). Proteasome inhibition in oxidative stress neurotoxicity: Implications for heat shock proteins. *J. Neurochem.* **77,** 1010–1017.

Doğan Ekici, A. I., Eren, B., Türkmen, N., Comunoğlu, N., and Fedakar, R. (2008). Clusterin expression in non-neoplastic adenohypophyses and pituitary adenomas: Cytoplasmic clusterin localization in adenohypophysis is related to aging. *Endocr. Pathol.* **19,** 47–53.

Dreher, D., and Junod, A. F. (1996). Role of oxygen free radicals in cancer development. *Eur. J. Cancer* **32A,** 30–38.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev.* **82,** 47–95.

Dröge, W. (2003). Oxidative stress and aging. *Adv. Exp. Med. Biol.* **543,** 191–200.

Dröge, W., and Schipper, H. M. (2007). Oxidative stress and aberrant signaling in aging and cognitive decline. *Aging Cell* **6,** 361–370.

Dumont, P., Burton, M., Chen, Q. M., Gonos, E. S., Frippiat, C., Mazarati, J. B., Eliaers, F., Remacle, J., and Toussaint, O. (2000). Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblast. *Free Radic. Biol. Med.* **28,** 361–373.

Dumont, P., Chainiaux, F., Eliaers, F., Petropoulou, C., Koch-Brandt, C., Gonos, E. S., and Toussaint, O. (2002). Overexpression of Apolipoprotein J in human fibroblasts protects against cytotoxicity and premature senescence induce by ethanol and tert-butylhydroperoxide. *Cell Stress Chaperones* **7,** 23–35.

Dyrks, T., Dyrks, E., Masters, C. L., and Beyreuther, K. (1993). Amyloidogenicity of rodent and human beta A4 sequences. *FEBS Lett.* **324,** 231–236.

Efthymiou, C. A., Mocanu, M. M., de Belleroche, J., Wells, D. J., Latchmann, D. S., and Yellon, D. M. (2004). Heat shock protein 27 protects the heart against myocardial infarction. *Basic Res. Cardiol.* **99,** 392–394.

Elchuri, S., Oberley, T. D., Qi, W., Eisenstein, R. S., Jackson Roberts, L., Van Remmen, H., Epstein, C. J., and Huang, T. T. (2005). CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene* **24,** 367–380.

Elpek, G. O., Karaveli, S., Simsek, T., Keles, N., and Aksoy, N. H. (2003). Expression of heat-shock proteins hsp27, hsp70 and hsp90 in malignant epithelial tumour of the ovaries. *Apmis* **111,** 523–530.

Farrow, B., and Evers, B. M. (2002). Inflammation and the development of pancreatic cancer. *Surg. Oncol.* **10,** 153–169.

Federico, A., Morgillo, F., Tuccillo, C., Ciardiello, F., and Loguercio, C. (2007). Chronic inflammation and oxidative stress in human carcinogenesis. *Int. J. Cancer* **121,** 2381–2386.

Finkel, T., and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature* **408,** 239–247.

Floyd, R. A., West, M. S., Eneff, K. L., Schneider, J. E., Wong, P. K., Tingey, D. T., and Hogsett, W. E. (1990). Conditions influencing yield and analysis of 8-hydroxy-20-deoxyguanosine in oxidatively damaged DNA. *Anal. Biochem.* **188,** 155–158.

Fraga, C. G., Shigenaga, M. K., Park, J. W., Degan, P., and Ames, B. N. (1990). Oxidative damage to DNA during aging: 8-hydroxy-20-deoxyguanosine in rat organ DNA and urine. *Proc. Natl. Acad. Sci. USA* **87,** 4533–4537.

Franceschi, C., and Bonafe, M. (2003). Centenarians as a model for healthy aging. *Biochem. Soc. Trans.* **31,** 457–461.

Francia, P., delli Gatti, C., Bachschmid, M., Martin-Padura, I., Savoia, C., Migliaccio, E., Pelicci, P. G., Schiavoni, M., Luscher, T. F., Volpe, M., and Cosentino, F. (2004). Deletion of p66shc gene protects against age-related endothelial dysfunction. *Circulation* **110,** 2889–2895.

Frippiat, C., Chen, Q. M., Zdanov, S., Magalhaes, J. P., Remacle, J., and Toussaint, O. (2001). Subcytotoxic H2O2 stress triggers a release of transforming growth factor-β1 which induces biomarkers of cellular senescence in human diploid fibroblasts. *J. Biol. Chem.* **276,** 2531–2537.

Garden, G. A., Bothwell, M., and Rubel, E. W. (1991). 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.* **22,** 590–604.

Golden, T. R., Hinerfeld, D. A., and Melov, S. (2002). Oxidative stress and aging: Beyond correlation. *Aging Cell* **2,** 117–123.

Graf, P. C., and Jakob, U. (2002). Redox-regulated molecular chaperones. *Cell. Mol. Life Sci.* **59,** 1624–16231.

Grune, T., Jung, T., Merker, K., and Davies, K. J. (2004). Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and "aggresomes" during oxidative stress, aging, and disease. *Int. J. Biochem. Cell Biol.* **36,** 2519–2530.

Gunter, M. J., and Leitzmann, M. F. (2006). Obesity and colorectal cancer: Epidemiology, mechanisms and candidate genes. *J. Nutr. Biochem.* **17,** 145–156.

Gupte, A., and Mumper, R. J. (2009). Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. *Cancer Treat. Rev.* **35,** 32–46.

Halazonetis, T. D., Gorgoulis, V. G., and Bartek, J. (2008). An oncogene-induced DNA damage model for cancer development. *Science* **319,** 1352–1355.

Halliwell, B. (2007). Oxidative stress and cancer: Have we moved forward? *Biochem. J.* **401,** 1–11.

Han, B. H., DeMattos, R. B., Dugan, L. L., Kim-Han, J. S., Brendza, R. P., Fryer, J. D., Kierson, M., Cirrito, J., Quick, K., Harmony, J. A., Aronow, B. J., and Holtzman, D. M. (2001). Clusterin contributes to caspase-3-independent brain injury following neonatal hypoxia-ischemia. *Nat. Med.* **7,** 338–743.

Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* **100,** 57–70.

Hardardóttir, I., Kunitake, S. T., Moser, A. H., Doerrler, W. T., Rapp, J. H., Grünfeld, C., and Feingold, K. R. (1994). Endotoxin and cytokines increase hepatic messenger RNA levels and serum concentrations of apolipoprotein J (clusterin) in Syrian hamsters. *J. Clin. Invest.* **94,** 1304–1309.

Hekimi, S., and Guarente, L. (2003). Genetics and the specificity of the aging process. *Science* **299,** 1351–1354.

Heller, A. R., Fiedler, F., Braun, P., Stehr, S. N., Bödeker, H., and Koch, T. (2003). Clusterin protects the lung from leukocyte-induced injury. *Shock* **20,** 166–170.

Hill, M. F., and Singal, P. K. (1997). Right and left myocardial antioxidant responses during heart failure subsequent to myocardial infarction. *Circulation* **96,** 2414–2420.

Ho, E., and Bray, T. M. (1999). Antioxidants,NFkappaB activation, and diabetogenesis. *Proc. Soc. Exp. Biol. Med.* **222,** 205–213.

Hoeller, C., Pratscher, B., Thallinger, C., Winter, D., Fink, D., Kovacic, B., Sexl, V., Wacheck, V., Gleave, M. E., Pehamberger, H., and Jansen, B. (2005). Clusterin regulates drug-resistance in melanoma cells. *J. Invest. Dermatol.* **124,** 1300–1307.

Hollander, J. M., Martin, J. L., Belke, D. D., Scott, B. T., Swanson, E., Krishnamoorthy, V., and Dillmann, W. H. (2004). Overexpression of wildtype heat shock protein 27 and a nonphosphorylatable heat shock protein 27 mutant protects against ischemia/reperfusion injury in a transgenic mouse model. *Circulation* **110,** 3544–3552.

Hough, C. D., Cho, K. R., Zonderman, A. B., Schwartz, D. R., and Morin, P. J. (2001). Coordinately up-regulated genes in ovarian cancer. *Cancer Res.* **61,** 3869–3876.

Humphreys, D., Hochgrebe, T. T., Easterbrook-Smith, S. B., Tenniswood, M. P., and Wilson, M. R. (1997). Effects of clusterin overexpression on TNFalpha- and TGFbeta-mediated death of L929 cells. *Biochemistry* **36,** 15233–15243.

Humphreys, D. T., Carver, J. A., Easterbrook-Smith, S. B., and Wilson, M. R. (1999). Clusterin has chaperone-like activity similar to that of small heat shock proteins. *J. Biol. Chem.* **274,** 6875–6881.

Ishikawa, T., Zhu, B. L., Li, D. R., Zhao, D., Michiue, T., and Maeda, H. (2006). Age-dependent increase of clusterin in the human pituitary gland. *Leg. Med. (Tokyo)* **8,** 28–33.

Itahana, Y., Piens, M., Sumida, T., Fong, S., Muschler, J., and Desprez, P. Y. (2007). Regulation of clusterin expression in mammary epithelial cells. *Exp. Cell Res.* **313,** 943–951.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic. Biol. Med.* **9,** 515–540.

Janig, E., Haslbeck, M., Aigelsreiter, A., Braun, N., Unterthor, D., Wolf, P., Khaskhely, N. M., Buchner, J., Denk, H., and Zatloukal, K. (2007). Clusterin associates with altered elastic fibers in human photoaged skin and prevents elastin from ultraviolet-induced aggregation *in vitro*. *Am. J. Pathol.* **171,** 1474–1482.

Jenne, D. E., and Tschopp, J. (1989). Molecular structure and functional characterization of a human complement cytolysis found in blood and seminal plasma: Identity to sulfated glycoprotein 2, a constituent of rat testis fluid. *Proc. Natl. Acad. Sci. USA* **86,** 7123–7127.

Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Ann. Neurol.* **53,** S26–S36.

Jin, G., and Howe, P. H. (1997). Regulation of clusterin gene expression by transforming growth factor α. *J. Biol. Chem.* **272,** 26620–26626.

Jo, H., Jia, Y., Subramanian, K. K., Hattori, H., and Luo, H. R. (2008). Cancer cell-derived clusterin modulates the phosphatidylinositol 3′-kinase-Akt pathway through attenuation of insulin-like growth factor 1 during serum deprivation. *Mol. Cell. Biol.* **28,** 4285–4299.

Jolly, C., and Morimoto, R. I. (2000). Role of the heat shock response and molecular chaperones in oncogenesis and cell death. *J. Natl Cancer Inst.* **92,** 1564–1572.

Kalenka, A., Feldmann, R. E., Jr., Otero, K., Maurer, M. H., Waschke, K. F., and Fiedler, F. (2006). Changes in the serum proteome of patients with sepsis and septic shock. *Anesth. Analg.* **103,** 1522–1526.

Kalka, K., Ahmad, N., Criswell, T., Boothman, D., and Mukhtar, H. (2000). Up-regulation of clusterin during phthalocyanine 4 photodynamic therapy-mediated apoptosis of tumor cells and ablation of mouse skin tumors. *Cancer Res.* **60,** 5984–5987.

Kamradt, M. C., Chen, F., and Cryns, V. L. (2001). The small heat shock protein alpha B-crystallin negatively regulates cytochrome *c*- and caspase-8-dependent activation of caspase-3 by inhibiting its autoproteolytic maturation. *J. Biol. Chem.* **276,** 16059–16063.

Kang, S. W., Lim, S. W., Choi, S. H., Shin, K. H., Chun, B. G., Park, I. S., and Min, B. H. (2000). Antisense oligonucleotide of clusterin mRNA induces apoptotic cell death and prevents adhesion of rat ASC-17D Sertoli cells. *Mol. Cells* **10,** 193–198.

Kang, Y. K., Hong, S. W., Lee, H., and Kim, W. H. (2004). Overexpression of clusterin in human hepatocellular carcinoma. *Hum. Pathol.* **35,** 1340–1346.

Kang, S. W., Shin, Y. J., Shim, Y. J., Jeong, S. Y., Park, I. S., and Min, B. H. (2005). Clusterin interacts with SCLIP (SCG10-like protein) and promotes neurite outgrowth of PC12 cells. *Exp. Cell Res.* **309,** 305–315.

Katsiki, M., Trougakos, I. P., Chondrogianni, N., Alexopoulos, E. C., Makropoulos, V., and Gonos, E. S. (2004). Alterations of senescence biomarkers in human cells by exposure to CrVI *in vivo* and *in vitro*. *Exp. Gerontol.* **39,** 1079–1087.

Kevans, D., Foley, J., Tenniswood, M., Sheahan, K., Hyland, J., O'Donoghue, D., Mulcahy, H., and O'Sullivan, J. (2009). High clusterin expression correlates with a poor outcome in stage II colorectal cancers. *Cancer Epidemiol. Biomarkers Prev.* **18,** 393–399.

Kida, E., Choi-Miura, N. H., and Wisniewski, K. E. (1995). Deposition of apolipoproteins E and J in senile plaques is topographically determined in both Alzheimer's disease and Down's syndrome brain. *Brain Res.* **685,** 211–216.

Kim, J. H., Kim, J. H., Yu, Y. S., Min, B. H., and Kim, K. W. (2007a). The role of clusterin in retinal development and free radical damage. *Br. J. Ophthalmol.* **91,** 1541–1546.

Kim, J. H., Yu, Y. S., Kim, J. H., Kim, K. W., and Min, B. H. (2007b). The role of clusterin in *in vitro* ischemia of human retinal endothelial cells. *Curr. Eye Res.* **32,** 693–698.

Kirkwood, T. B. (2002). Evolution of ageing. *Mech. Ageing Dev.* **123,** 737–745.

Klatt, P., Molina, E. P., DeLacoba, M. G., Padilla, C. A., Martinez-Galesto, E., Barcena, J. A., and Lamas, S. (1999). Redox regulation of c-Jun binding by reversible glutathiolation. *Faseb J.* **13,** 1481–1490.

Klaunig, J. E., and Kamendulis, L. M. (2004). The role of oxidative stress in carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* **44,** 239–267.

Klokov, D., Criswell, T., Leskov, K. S., Araki, S., Mayo, L., and Boothman, D. A. (2004). IR-inducible clusterin gene expression: A protein with potential roles in ionizing radiation-induced adaptive responses, genomic instability, and bystander effects. *Mutat. Res.* **568,** 97–110.

Kondo, S., Toyokuni, S., Iwasa, Y., Tanaka, T., Onodera, H., Hiai, H., and Imamura, M. (1999). Persistent oxidative stress in human colorectal carcinoma, but not adenoma. *Free Radic. Biol. Med.* **27,** 401–410.

Krijnen, P. A., Cillessen, S. A., Manoe, R., Muller, A., Visser, C. A., Meijer, C. J., Musters, R. J., Hack, C. E., Aarden, L. A., and Niessen, H. W. (2005). Clusterin: A protective mediator for ischemic cardiomyocytes? *Am. J. Physiol. Heart Circ. Physiol.* **289,** H2193–H2202.

Lam, Y. W., Tam, N. N., Evans, J. E., Green, K. M., Zhang, X., and Ho, S. M. (2008). Differential proteomics in the aging Noble rat ventral prostate. *Proteomics* **8,** 2750–2763.

Lane, D. P. (1992). Cancer. p53, guardian of the genome. *Nature* **358,** 15–16.

Lane, N. (2003). A unifying view of ageing and disease: The doubleagent theory. *J. Theor. Biol.* **225,** 531–540.

Lau, K. M., Tam, N. N., Thompson, C., Cheng, R. Y., Leung, Y. K., and Ho, S. M. (2003). Age-associated changes in histology and gene-expression profile in the rat ventral prostate. *Lab. Invest.* **83,** 743–757.

Lau, S. H., Sham, J. S., Xie, D., Tzang, C. H., Tang, D., Ma, N., Hu, L., Wang, Y., Wen, J. M., Xiao, G., Zhang, W. M., Lau, G. K., *et al.* (2006). Clusterin plays an important role in hepatocellular carcinoma metastasis. *Oncogene* **25,** 1242–1250.

Leskov, K. S., Klokov, D. Y., Li, J., Kinsella, T. J., and Boothman, D. A. (2003). Synthesis and functional analysis of nuclear clusterin: A cell death protein. *J. Biol. Chem.* **278,** 11590–11600.

Li, R. K., Sole, M. J., Mickle, D. A., Schimmer, J., and Goldstein, D. (1998). Vitamin E and oxidative stress in the heart of the cardiomyopathic syrian hamster. *Free Radic. Biol. Med.* **24,** 252–258.

Li, X., Massa, P. E., Hanidu, A., Peet, G. W., Aro, P., Savitt, A., Mische, S., Li, J., and Marcu, K. B. (2002). IKKalpha, IKKbeta, and NEMO/IKKgamma are each required for the NF-kappa B-mediated inflammatory response program. *J. Biol. Chem.* **277,** 45129–45140.

Li, Y., Sagar, M. B., Wassler, M., Shelat, H., and Geng, Y. J. (2007). Apolipoprotein-J prevention of fetal cardiac myoblast apoptosis induced by ethanol. *Biochem. Biophys. Res. Commun.* **357,** 157–161.

Liu, S., Li, J., Tao, Y., and Xiao, X. (2007). Small heat shock protein alphaB-crystallin binds to p53 to sequester its translocation to mitochondria during hydrogen peroxide-induced apoptosis. *Biochem. Biophys. Res. Commun.* **354,** 109–114.

Liu, T., Liu, P. Y., Tee, A. E., Haber, M., Norris, M. D., Gleave, M. E., and Marshall, G. M. (2009). Over-expression of clusterin is a resistance factor to the anti-cancer effect of histone deacetylase inhibitors. *Eur. J. Cancer* **45,** 1846–1854.

Loft, S., and Poulsen, H. E. (1996). Cancer risk and oxidative DNA damage in man. *J. Mol. Med. JMM* **74,** 297–312.

Loison, F., Debure, L., Nizard, P., le Goff, P., Michel, D., and le Drean, Y. (2006). Up-regulation of the clusterin gene after proteotoxic stress: Implication of HSF1-HSF2 heterocomplexes. *Biochem. J.* **395,** 223–231.

Lourda, M., Trougakos, I. P., and Gonos, E. S. (2007). Development of resistance to chemotherapeutic drugs in human osteosarcoma cell lines largely depends on up-regulation of clusterin/apolipoprotein J. *Int. J. Cancer* **120,** 611–622.

Lund, P., Weisshaupt, K., Mikeska, T., Jammas, D., Chen, X., Kuban, R. J., Ungethüm, U., Krapfenbauer, U., Herzel, H. P., Schäfer, R., Walter, J., and Sers, C. (2006). Oncogenic HRAS suppresses clusterin expression through promoter hypermethylation. *Oncogene* **25,** 4890–4903.

Mackness, B., Hunt, R., Durrington, P. N., and Mackness, M. I. (1997). Increased immunolocalization of paraoxonase, clusterin, and apolipoprotein A-I in the human artery wall with the progression of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **17,** 1233–1338.

Mancuso, C., Bates, T. E., Butterfield, D. A., Calafato, S., Cornelius, C., De Lorenzo, A., Dinkova Kostova, A. T., and Calabrese, V. (2007). Natural antioxidants in Alzheimer's disease. *Expert Opin. Investig. Drugs* **16,** 1921–1931.

Mantovani, A. (2009). Cancer: Inflaming metastasis. *Nature* **457,** 36–37.

Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008). Cancer-related inflammation. *Nature* **454,** 436–444.

Mao, Y. W., Liu, J. P., Xiang, H., and Li, D. W. (2004). Human alphaA- and alphaB-crystallins bind to Bax and Bcl-X(S) to sequester their translocation during staurosporine-induced apoptosis. *Cell Death Differ.* **11,** 512–526.

Mariani, E., Polidori, M. C., Cherubini, A., and Mecocci, P. (2005). Oxidative stress in brain aging, neurodegenerative and vascular diseases: An overview. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **827,** 65–75.

Martindale, J. L., and Holbrook, N. J. (2002). Cellular response to oxidative stress: Signaling for suicide and survival. *J. Cell Physiol.* **192,** 1–15.

Mates, J. M., Perez-Gomez, C., and De Castro, I. N. (1999). Antioxidant enzymes and human diseases. *Clin. Biochem.* **32,** 595–603.

Matsubara, E., Soto, C., Governale, S., Frangione, B., and Ghiso, J. (1996). Apolipoprotein J and Alzheimer's amyloid beta solubility. *Biochem. J.* **316,** 671–679.

McCall, M. R., and Frei, B. (1999). Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radic. Biol. Med.* **26,** 1034–1053.

McHattie, S., and Edington, N. (1999). Clusterin prevents aggregation of neuropeptide 106–126 *in Vitro*. *Biochem. Biophys. Res. Commun.* **259,** 336–340.

McLaughlin, L., Zhu, G., Mistry, M., Ley-Ebert, C., Stuart, W. D., Florio, C. J., Groen, P. A., Witt, S. A., Kimball, T. R., Witte, D. P., Harmony, J. A., and Aronow, B. J. (2000). Apolipoprotein J/clusterin limits the severity of murine autoimmune myocarditis. *J. Clin. Invest.* **106,** 1105–1113.

Michel, D., Chatelain, G., Herault, Y., and Brun, G. (1995). The expression of the avian clusterin gene can be driven by two alternative promoters with distinct regulatory elements. *Eur. J. Biochem.* **229,** 215–223.

Migliaccio, E., Giorgio, M., Mele, S., Pelicci, G., Reboldi, P., Pandolfi, P. P., Lanfrancone, L., and Pelicci, P. G. (1999). The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* **402,** 309–313.

Minelli, A., Bellezza, I., Conte, C., and Culig, Z. (2009). Oxidative stress-related aging: A role for prostate cancer? *Biochim. Biophys. Acta* **1795,** 83–91.

Miyake, H., Chi, K. N., and Gleave, M. E. (2000a). Antisense TRPM-2 oligodeoxynucleotides chemosensitize human androgen-independent PC-3 prostate cancer cells both *in vitro* and *in vivo*. *Clin. Cancer Res.* **6,** 1655–1663.

Miyake, H., Nelson, C., Rennie, P. S., and Gleave, M. E. (2000b). Acquisition of chemoresistant phenotype by overexpression of the antiapoptotic gene testosterone-repressed prostate message-2 in prostate cancer xenograft models. *Cancer Res.* **60,** 2547–2554.

Miyake, H., Nelson, C., Rennie, P. S., and Gleave, M. E. (2000c). Testosterone-repressed prostate message-2 is an antiapoptotic gene involved in progression to androgen independence in prostate cancer. *Cancer Res.* **60,** 170–176.

Miyake, H., Hara, I., Gleave, M. E., and Eto, H. (2004). Protection of androgen-dependent human prostate cancer cells from oxidative stress-induced DNA damage by overexpression of clusterin and its modulation by androgen. *Prostate* **61,** 318–323.

Mollah, Z. U., Pai, S., Moore, C., O'Sullivan, B. J., Harrison, M. J., Peng, J., Phillips, K., Prins, J. B., Cardinal, J., and Thomas, R. (2008). Abnormal NF-{kappa}B function characterizes human type 1 diabetes dendritic cells and monocytes. *J. Immunol.* **180,** 3166–3175.

Moretti, R. M., Marelli, M. M., Mai, S., Cariboni, A., Scaltriti, M., Bettuzzi, S., and Limonta, P. (2007). Clusterin isoforms differentially affect growth and motility of prostate cells: Possible implications in prostate tumorigenesis. *Cancer Res.* **67,** 10325–10333.

Morimoto, R. I. (2002). Dynamic remodeling of transcription complexes by molecular chaperones. *Cell* **110,** 281–284.

Moulson, C. L., and Millis, A. J. (1999). Clusterin (Apo J) regulates vascular smooth muscle cell differentiation *in vitro*. *J. Cell. Physiol.* **180,** 355–364.

Mourra, N., Couvelard, A., Tiret, E., Olschwang, S., and Flejou, J. F. (2007). Clusterin is highly expressed in pancreatic endocrine tumours but not in solid pseudopapillary tumours. *Histopathology* **50,** 331–337.

Nizard, P., Tetley, S., Le Dréan, Y., Watrin, T., Le Goff, P., Wilson, M. R., and Michel, D. (2007). Stress-induced retrotranslocation of clusterin/ApoJ into the cytosol. *Traffic* **8,** 554–565.

O'Bryan, M. K., Cheema, S. S., Bartlett, P. F., Murphy, B. F., and Pearse, M. J. (1993). Clusterin levels increase during neuronal development. *J. Neurobiol.* **2,** 421–432.

Oda, T., Pasinetti, G. M., Osterburg, H. H., Anderson, C., Johnson, S. A., and Finch, C. E. (1994). Purification and characterization of brain clusterin. *Biochem. Biophys. Res. Commun.* **204,** 1131–1136.

Oda, T., Wals, P., Osterburg, H. H., Johnson, S. A., Pasinetti, G. M., Morgan, T. E., Rozovsky, I., Stine, W. B., Snyder, S. W., Holzman, T. F., Krafft, G. A., and Finch, C. E. (1995). Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (Abeta 1–42) and forms slowly sedimenting A beta complexes that cause oxidative stress. *Exp. Neurol.* **136,** 22–31.

Omwancha, J., Anway, M. D., and Brown, T. R. (2009). Differential age-associated regulation of clusterin expression in prostate lobes of brown Norway rats. *Prostate* **69,** 115–125.

O'Sullivan, J., Whyte, L., Drake, J., and Tenniswood, M. (2003). Alterations in the post-translational modification and intracellular trafficking of clusterin in MCF-7 cells during apoptosis. *Cell Death Differ.* **10,** 914–927.

Ouyang, X. S., Wang, X., Lee, D. T. W., Tsao, S. W., and Wong, Y. C. (2001). Up-regulation of TRPM-2, MMP-7 and ID-1 during sex hormone-induced prostate carcinogenesis in the Noble rat. *Carcinogenesis* **22,** 965–973.

Parczyk, K., Pilarsky, C., Rachel, U., and Koch-Brandt, C. (1994). Gp80 (clusterin; TRPM-2) mRNA level is enhanced in human renal clear cell carcinomas. *J. Cancer Res. Clin. Oncol.* **120,** 186–188.

Park, Y. M., Han, M. Y., Blackburn, R. V., and Lee, Y. J. (1998). Overexpression of HSP25 reduces the level of TNF alpha-induced oxidative DNA damage biomarker, 8-hydroxy-20-deoxyguanosine, in L929 cells. *J. Cell. Physiol.* **174,** 27–34.

Park, D. C., Yeo, S. G., Wilson, M. R., Yerbury, J. J., Kwong, J., Welch, W. R., Choi, Y. K., Birrer, M. J., Mok, S. C., and Wong, K. K. (2008). Clusterin interacts with Paclitaxel and confer Paclitaxel resistance in ovarian cancer. *Neoplasia* **10,** 964–972.

Partheen, K., Levan, K., Osterberg, L., Claesson, I., Fallenius, G., Sundfeldt, K., and Horvath, G. (2008). Four potential biomarkers as prognostic factors in stage III serous ovarian adenocarcinomas. *Int. J. Cancer* **123,** 2130–2137.

Patel, N. V., Wei, M., Wong, A., Finch, C. E., and Morgan, T. E. (2004). Progressive changes in regulation of apolipoproteins E and J in glial cultures during postnatal development and aging. *Neurosci. Lett.* **371,** 199–204.

Patterson, S. G., Wei, S., Chen, X., Sallman, D. A., Gilvary, D. L., Zhong, B., Pow-Sang, J., Yeatman, T., and Djeu, J. Y. (2006). Novel role of Stat1 in the development of docetaxel resistance in prostate tumor cells. *Oncogene* **25,** 6113–6122.

Paul, C., Manero, F., Gonin, S., Kretz-Remy, C., Virot, S., and Arrigo, A. P. (2002). Hsp27 as a negative regulator of cytochrome C release. *Mol. Cell. Biol.* **22,** 816–834.

Petropoulou, C., Trougakos, I. P., Kolettas, E., Toussaint, O., and Gonos, E. S. (2001). Clusterin/apolipoprotein J is a novel biomarker of cellular senescence, that does not affect the proliferative capacity of human diploid fibroblasts. *FEBS Lett.* **509,** 287–297.

Pirkkala, L., Nykanen, P., and Sistonen, L. (2001). Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. *Faseb J.* **15,** 1118–1131.

Poon, S., Easterbrook-Smith, S. B., Rybchyn, M. S., Carver, J. A., and Wilson, M. R. (2000). Clusterin is an ATP-independent chaperone with very broad substrate specificity that stabilizes stressed proteins in a folding-competent state. *Biochemistry* **39,** 15953–15960.

Poppek, D., and Grune, T. (2006). Proteasomal defense of oxidative protein modifications. *Antioxid. Redox Signal.* **8,** 173–184.

Preville, X., Salvemini, F., Giraud, S., Chaufour, S., Paul, C., Stepien, G., Ursini, M. V., and Arrigo, A. P. (1999). Mammalian small stress proteins protect against oxidative stress through their ability to increase glucose-6-phosphate dehydrogenase activity and by maintaining optimal cellular detoxifying machinery. *Exp. Cell Res.* **247,** 61–78.

Pucci, S., Bonanno, E., Pichiorri, F., Angeloni, C., and Spagnoli, L. G. (2004). Modulation of different clusterin isoforms in human colon tumorigenesis. *Oncogene* **23,** 2298–2304.

Pucci, S., Bonanno, E., Sesti, F., Mazzarelli, P., Mauriello, A., Ricci, F., Zoccai, G. B., Rulli, F., Galatà, G., and Spagnoli, L. G. (2009). Clusterin in stool: A new biomarker for colon cancer screening? *Am. J. Gastroenterol.* [Epub ahead of print].

Rastaldi, M.P, Candiano, G., Musante, L., Bruschi, M., Armelloni, S., Rimoldi, L., Tardanico, R., Sanna-Cherchi, S., Ferrario, F., Montinaro, V., Haupt, R., Parodi, S., *et al.* (2006). Glomerular clusterin is associated with PKC-alpha/beta regulation and good outcome of membranous glomerulonephritis in humans. *Kidney Int.* **70,** 477–485.

Ray, P. S., Martin, J. L., Swanson, E. A., Otani, H., Dillmann, W. H., and Das, D. K. (2001). Transgene overexpression of aB crystallin confers simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion. *Faseb J.* **15,** 393–402.

Reddy, K. B., Jin, G., Karode, M. C., Harmony, J. A. K., and Howe, P. H. (1996). Transforming growth factor β (TGFβ)-induced nuclear localization of Apolipoprotein J/Clusterin in epithelial cells. *Biochemistry* **35,** 6157–6163.

Redondo, M., Villar, E., Torres-Munoz, J., Tellez, T., Morell, M., and Petito, C. K. (2002). Overexpression of clusterin in human breast carcinoma. *Am. J. Pathol.* **157,** 393–399.

Renkawek, K., Voorter, C. E., Bosman, G. J., van Workum, F. P., and de Jong, W. W. (1994). Expression of aBcrystallin in Alzheimer's disease. *Acta Neuropathol. (Berl.)* **87,** 155–160.

Ricci, F., Pucci, S., Sesti, F., Missiroli, F., Cerulli, L., and Spagnoli, L. G. (2007). Modulation of Ku70/80, clusterin/ApoJ isoforms and Bax expression in indocyanine-green-mediated photooxidative cell damage. *Ophthalmic Res.* **39,** 164–173.

Rodríguez-Piñeiro, A. M., de la Cadena, M. P., López-Saco, A., and Rodríguez-Berrocal, F. J. (2006). Differential expression of serum clusterin isoforms in colorectal cancer. *Mol. Cell. Proteomics* **5,** 1647–1657.

Rosenberg, M. E., Girton, R., Finkel, D., Chmielewski, D., Barrie, A.,, III, Witte, D. P., Zhu, G., Bissler, J. J., Harmony, J. A., and Aronow, B. J. (2002). Apolipoprotein J/clusterin prevents a progressive glomerulopathy of aging. *Mol. Cell. Biol.* **22,** 1893–1902.

Saffer, H., Wahed, A., Rassidakis, G. Z., and Medeiros, L. J. (2002). Clusterin expression in malignant lymphomas. *Mod. Pathol.* **15,** 1221–1223.

Sagi, O., Wolfson, M., Utko, N., Muradian, K., and Fraifeld, V. (2005). p66ShcA and ageing: Modulation by longevity-promoting agent aurintricarboxylic acid. *Mech. Ageing Dev.* **126,** 249–254.

Sallman, D. A., Chen, X., Zhong, B., Gilvary, D. L., Zhou, J., Wei, S., and Djeu, J. Y. (2007). Clusterin mediates TRAIL resistance in prostate tumor cells. *Mol. Cancer Ther.* **6,** 2938–2947.

Santilli, G., Aronow, B. J., and Sala, A. (2003). Essential requirement of apolipoprotein J (clusterin) signaling for IkappaB expression and regulation of NF-kappaB activity. *J. Biol. Chem.* **278,** 38214–38219.

Saradha, B., Vaithinathan, S., and Mathur, P. P. (2008). Lindane alters the levels of HSP70 and clusterin in adult rat testis. *Toxicology* **243,** 116–123.

Sarkar, F. H., Li, Y., Wang, Z., and Kong, D. (2008). NF-kappaB signaling pathway and its therapeutic implications in human diseases. *Int. Rev. Immunol.* **27,** 293–319.

Saura, J., Petegnief, V., Wu, X., Liang, Y., and Paul, S. M. (2003). Microglial apolipoprotein E and astroglial apolipoprotein J expression *in vitro*: Opposite effects of lipopolysaccharide. *J. Neurochem.* **85,** 1455–1467.

Savković, V., Gantzer, H., Reiser, U., Selig, L., Gaiser, S., Sack, U., Klöppel, G., Mössner, J., Keim, V., Horn, F., and Bödeker, H. (2007). Clusterin is protective in pancreatitis through anti-apoptotic and anti-inflammatory properties. *Biochem. Biophys. Res. Commun.* **356,** 431–437.

Scaltriti, M., Brausi, M., Amorosi, A., Caporali, A., D'Arca, D., Astancolle, S., Corti, A., and Bettuzzi, S. (2004a). Clusterin (SGP-2, ApoJ) expression is downregulated in low- and high-grade human prostate cancer. *Int. J. Cancer* **108,** 23–30.

Scaltriti, M., Santamaria, A., Paciucci, R., and Bettuzzi, S. (2004b). Intracellular clusterin induces G2-M phase arrest and cell death in PC-3 prostate cancer cells1. *Cancer Res.* **64,** 6174–6182.

Schlabach, M. R., Luo, J., Solimini, N. L., Hu, G., Xu, Q., Li, M. Z., Zhao, Z., Smogorzewska, A., Sowa, M. E., Ang, X. L., Westbrook, T. F., Liang, A. C., *et al.* (2008). Cancer proliferation gene discovery through functional genomics. *Science* **319,** 620–624.

Schutte, J., Minna, J. D., and Birer, M. I. (1989). Deregulated expression of human c-jun transforms primary rat embryo cells in cooperation with an activated c-Ha-ras gene and transforms rat-la cells as a single gene. *Proc. Natl. Acad. Sci. USA* **86,** 2257–2261.

Schwarz, M., Spath, L., Lux, C. A., Paprotka, K., Torzewski, M., Dersch, K., Koch-Brandt, C., Husmann, M., and Bhakdi, S. (2008). Potential protective role of apoprotein J (clusterin) in atherogenesis: Binding to enzymatically modified low-density lipoprotein reduces fatty acid-mediated cytotoxicity. *Thromb. Haemost.* **100,** 110–118.

Schwochau, G. B., Nath, K. A., and Rosenberg, M. E. (1998). Clusterin protects against oxidative stress *in vitro* through aggregative and nonaggregative properties. *Kidney Int.* **53,** 1647–1653.

Sciarra, A., Mariotti, G., Salciccia, S., Gomez, A. A., Monti, S., Toscano, V., and Di Silverio, F. (2008). Prostate growth and inflammation. *Steroid Biochem. Mol. Biol.* **108,** 254–260.

Sensibar, J. A., Sutkowski, D. M., Raffo, A., Buttyan, R., Griswold, M. D., Sylvester, S. R., Kozlowski, J. M., and Lee, C. (1995). Prevention of cell death induced by tumor necrosis factor alpha in LNCaP cells by overexpression of sulfated glycoprotein-2 (clusterin). *Cancer Res.* **55,** 2431–2437.

Senthil, K., Aranganathan, S., and Nalini, N. (2004). Evidence of oxidative stress in the circulation of ovarian cancer patients. *Clin. Chim. Acta* **339,** 27–32.

Shackelford, R. E., Kaufmann, W. K., and Paules, R. S. (2000). Oxidative stress and cell cycle checkpoint function. *Free Radic. Biol. Med.* **28,** 1387–1404.

Shannan, B., Seifert, M., Leskov, K., Willis, J., Boothman, D., Tilgen, W., and Reichrath, J. (2006). Challenge and promise: Roles for clusterin in pathogenesis, progression and therapy of cancer. *Cell Death Differ.* **13,** 12–19.

Sharma, A., Bhattacharya, B., Puri, R. K., and Maheshwari, R. K. (2008). Venezuelan equine encephalitis virus infection causes modulation of inflammatory and immune response genes in mouse brain. *BMC Genomics* **9,** 289.

Shaulian, E., and Karin, M. (2001). AP-1 in cell proliferation and survival. *Oncogene* **20,** 2390–2400.

Shim, Y. J., Shin, Y. J., Jeong, S. Y., Kang, S. W., Kim, B. M., Park, I. S., and Min, B. H. (2009). Epidermal growth factor receptor is involved in clusterin-induced astrocyte proliferation. *Neuroreport* **20,** 435–439.

Shinohara, H., Inaguma, Y., Goto, S., Inagaki, T., and Kato, K. (1993). aB crystallin and HSP28 are enhanced in the cerebral cortex of patients with Alzheimer's disease. *J. Neurol. Sci.* **119,** 203–208.

Singh, B. N., Rao, K. S., Ramakrishna, T., Rangaraj, N., and Rao, Ch. M. (2007). Association of alphaB-crystallin, a small heat shock protein, with actin: Role in modulating actin filament dynamics *in vivo*. *J. Mol. Biol.* **366,** 756–767.

Sintich, S. M., Steinberg, J., Kozlowski, J. M., Lee, C., Pruden, S., Sayeed, S., and Sensibar, J. A. (1999). Cytotoxic sensitivity to tumor necrosis factor-alpha in PC3 and LNCaP prostatic cancer cells is regulated by extracellular levels of SGP-2 (clusterin). *Prostate* **39,** 87–93.

Song, H., Zhang, B., Watson, M. A., Humphrey, P. A., Lim, H., and Milbrandt, J. (2009). Loss of Nkx3.1 leads to the activation of discrete downstream target genes during prostate tumorigenesis. *Oncogene* [Epub ahead of print].

Soti, C., and Csermely, P. (2003). Aging and molecular chaperones. *Exp. Gerontol.* **38,** 1037–1040.

Soti, C., Pal, C., Papp, B., and Csermely, P. (2005). Molecular chaperones as regulatory elements of cellular networks. *Curr. Opin. Cell Biol.* **17,** 210–215.

Stadtman, E. R. (1992). Protein oxidation and aging. *Science* **257,** 1220–1224.

Steinberg, J., Oyasu, R., Lang, S., Sintich, S., Rademaker, A., Lee, C., Kozlowski, J. M., and Sensibar, J. A. (1997). Intracellular levels of SGP-2 (Clusterin) correlate with tumor grade in prostate cancer. *Clin. Cancer Res.* **3,** 1707–1711.

Stocker, R., and Keaney, J. F., Jr. (2004). Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* **84,** 1381–1478.

Storz, P. (2005). Reactive oxygen species in tumor progression. *Front Biosci.* **10,** 1881–1896.

Strocchi, P., Smith, M. A., Perry, G., Tamagno, E., Danni, O., Pession, A., Gaiba, A., and Dozza, B. (2006). Clusterin up-regulation following sub-lethal oxidative stress and lipid peroxidation in human neuroblastoma cells. *Neurobiol. Aging* **27,** 1588–1594.

Su, C. Y., Chong, K. Y., Edelstein, K., Lille, S., Khardori, R., and Lai, C. C. (1999). Constitutive hsp70 attenuates hydrogen peroxide-induced membrane lipid peroxidation. *Biochem. Biophys. Res. Commun.* **265,** 279–284.

Sun, Y., and MacRae, T. H. (2005). Small heat shock proteins: Molecular structure and chaperone function. *Cell. Mol. Life Sci.* **62,** 2460–2476.

Swertfegerv, D. K., Witte, D. P., Stuart, W. D., Rockman, H. A., and Harmony, J. A. (1996). Apolipoprotein J/clusterin induction in myocarditis: A localized response gene to myocardial injury. *Am. J. Pathol.* **148,** 1971–1983.

Takase, O., Minto, A. W., Puri, T. S., Cunningham, P. N., Jacob, A., Hayashi, M., and Quigg, R. J. (2008). Inhibition of NF-kappaB-dependent Bcl-xL expression by clusterin promotes albumin-induced tubular cell apoptosis. *Kidney Int.* **73,** 567–577.

Thomàs-Moyà, E., Gianotti, M., Proenza, A. M., and Lladó, I. (2006). The age-related paraoxonase 1 response is altered by long-term caloric restriction in male and female rats. *J. Lipid Res.* **47,** 2042–2048.

Thomas-Tikhonenko, A., Viard-Leveugle, I., Dews, M., Wehrli, P., Sevignani, C., Yu, D., Ricci, S., el-Deiry, W., Aronow, B., Kaya, G., Saurat, J. H., and French, L. E. (2004). Myc-transformed epithelial cells down-regulate clusterin, which inhibits their growth *in vitro* and carcinogenesis *in vivo*. *Cancer Res.* **64,** 3126–3136.

Toyokuni, S. (2008). Molecular mechanisms of oxidative stress-induced carcinogenesis: From epidemiology to oxygenomics. *IUBMB Life* **60,** 441–447.

Trougakos, I. P., and Gonos, E. S. (2002). Clusterin/Apolipoprotein J in human aging and cancer. *Int. J. Biochem. Cell Biol.* **34,** 1430–1448.

Trougakos, I. P., and Gonos, E. S. (2006). 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.* **40,** 1324–1334.

Trougakos, I. P., Chondrogianni, N., Pimenidou, A., Katsiki, M., Tzavelas, C., and Gonos, E. S. (2002a). Slowing down cellular aging *in vitro*. *In* "Modulating Aging and Longevity," (S. I. Rattan, Ed.). pp. 65–83. Kluwer Academic Publishers, The Netherlands.

Trougakos, I. P., Poulakou, M., Stathatos, M., Chalikia, A., Melidonis, A., and Gonos, E. S. (2002b). Serum levels of the senescence biomarker Clusterin/Apolipoprotein J increase significantly in diabetes type II and during development of coronary heart disease or at myocardial infarction. *Exp. Gerontol.* **37,** 1175–1187.

Trougakos, I. P., So, A., Jansen, B., Gleave, M. E., and Gonos, E. S. (2004). Silencing expression of the Clusterin/Apolipoprotein J gene in human cancer cells using small interfering RNA induces spontaneous apoptosis, reduced growth ability and cell sensitization to genotoxic and oxidative stress. *Cancer Res.* **64,** 1834–1842.

Trougakos, I. P., Lourda, M., Agiostratidou, G., Kletsas, D., and Gonos, E. S. (2005). Differential effects of Clusterin/Apolipoprotein J on cellular growth and survival. *Free Radic. Biol. Med.* **38,** 436–449.

Trougakos, I. P., Pawelec, G., Tzavelas, C., Ntouroupi, T., and Gonos, E. S. (2006a). Clusterin/Apolipoprotein J up-regulation after Zinc exposure, replicative senescence or differentiation of human haematopoietic cells. *Biogerontology* **7,** 375–382.

Trougakos, I. P., Petropoulou, C., Franceschi, C., and Gonos, E. S. (2006b). Reduced expression levels of the senescence biomarker Clusterin/Apolipoprotein J in lymphocytes from healthy centenarians. *Ann. N. Y. Acad. Sci.* **1067,** 294–300.

Trougakos, I. P., Djeu, J. Y., Gonos, E. S., and Boothman, D. A. (2009a). Advances and challenges in basic and translational research on clusterin. *Cancer Res.* **69,** 403–406.

Trougakos, I. P., Lourda, M., Antonelou, M. H., Kletsas, D., Gorgoulis, V. G., Papassideri, I. S., Zou, Y., Margaritis, L. H., Boothman, D. A., and Gonos, E. S. (2009b). Intracellular Clusterin inhibits mitochondrial apoptosis by suppressing p53-activating stress signals and stabilizing the cytosolic Ku70–Bax protein complex. *Clin. Cancer Res.* **15,** 48–59.

Tycko, B., Feng, L., Nguyen, L., Francis, A., Hays, A., Chung, W-Y., Tang, M-X., Stern, Y., Sahota, A., Hendrie, H., and Mayeux, R. (1996). Polymorphisms in the human Apolipoprotein J/Clusterin gene: Ethnic variation and distribution in Alzheimer's disease. *Hum. Genet.* **98,** 430–436.

Ungar, D. R., Hailat, N., Strahler, J. R., Kuick, R. D., Brodeur, G. M., Seeger, R. C., Reynolds, C. P., and Hanash, S. M. (1994). Hsp27 expression in neuroblastoma: Correlation with disease stage. *J. Natl Cancer Inst.* **86,** 780–784.

Upham, B. L., Sun Kang, K., Cho, H-Y., and Trosko, J. E. (1997). Hydrogen peroxide inhibits gap junctional intercellular communication in glutathione sufficient but not glutathione deficient cells. *Carcinogenesis* **18,** 37–42.

Vaithinathan, S., Saradha, B., and Mathur, P. P. (2009). Methoxychlor an organochlorine pesticide-induced alteration in the levels of HSP70 and clusterin is accompanied with oxidative stress in adult rat testis. *J. Biochem. Mol. Toxicol.* **23,** 29–35.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., and Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* **160,** 1–40.

Viard, I., Wehrli, P., Jornot, L., Bullani, R., Vechietti, J- L., Schifferli, J. A., Tschopp, J., and French, L. E. (1999). Clusterin gene expression mediates resistance to apoptotic cell death induced by heat shock and oxidative stress. *J. Invest. Dermatol.* **112,** 290–296.

Vousden, K. H., and Lane, D. P. (2007). p53 in health and disease. *Nat. Rev. Mol. Cell. Biol.* **8,** 275–283.

Wehrli, P., Charnay, Y., Vallet, P., Zhu, G., Harmony, J., Aronow, B., Tschopp, J., Bouras, C., Viard-Leveugle, I., French, L. E., and Giannakopoulos, P. (2001). Inhibition of post-ischemic brain injury by clusterin overexpression. *Nat. Med.* **7,** 977–978.

Weinberg, R. A. (2008). Mechanisms of malignant progression. *Carcinogenesis* **29,** 1092–1095.

Weitzman, S. A., Turk, P. W., Milkowski, D. H., and Kozlowski, K. (1994). Free radical adducts induce alterations in DNA cytosine methylation. *Proc. Natl. Acad. Sci. USA* **91,** 1261–1264.

Wellmann, A., Thieblemont, C., Pittaluga, S., Sakai, A., Jaffe, E. S., Siebert, P., and Raffeld, M. (2000). Detection of differentially expressed genes in lymphomas using cDNA arrays: Identification of clusterin as a new diagnostic marker for anaplastic large-cell lymphomas. *Blood* **96,** 398–404.

Wilson, M. R., and Easterbrook-Smith, S. B. (2000). Clusterin is a secreted mammalian chaperone. *Trends Biochem. Sci.* **25,** 95–98.

Wong, P., Taillefer, D., Lakins, J., Pineault, J., Chader, G., and Tenniswood, M. (1994). Molecular characterization of human TRPM-2/clusterin, a gene associated with sperm maturation, apoptosis and neurodegeneration. *Eur. J. Biochem.* **221,** 917–925.

Xie, D., Sham, J. S., Zeng, W. F., Che, L. H., Zhang, M., Wu, H. X., Lin, H. L., Wen, J. M., Lau, S. H., Hu, L., and Guan, X. Y. (2005). Oncogenic role of clusterin overexpression in multistage colorectal tumorigenesis and progression. *World J. Gastroenterol.* **11,** 3285–3289.

Yamamoto, H., Yamamoto, Y., Yamagami, K., Kume, M., Kimoto, S., Toyokuni, S., Uchida, K., Fukumoto, M., and Yamaoka, Y. (2000). Heat-shock preconditioning reduces oxidative protein denaturation and ameliorates liver injury by carbon tetrachloride in rats. *Res. Exp. Med. (Berl.)* **199,** 309–318.

Yang, C- R., Leskov, K., Hosley-Eberlein, K., Criswell, T., Pink, J. J., Kinsella, T. J., and Boothman, D. A. (2000). Nuclear clusterin/XIP8, an x-rayinduced Ku70-binding protein that signals cell death. *Proc. Natl. Acad. Sci. USA* **97,** 5907–5912.

Yeh, C-C., Hou, M-F., Tsai, S-M., Lin, S-K., Hsiao, J-K., Huang, J-C., Wang, L. H., Wu, S. H., Hou, L. A., Ma, H., and Tsai, L. Y. (2005). Superoxide anion radical, lipid peroxides and antioxidant status in the blood of patients with breast cancer. *Clin. Chim. Acta* **361,** 101–111.

Yerbury, J. J., Poon, S., Meehan, S., Thompson, B., Kumita, J. R., Dobson, C. M., and Wilson, M. R. (2007). The extracellular chaperone clusterin influences amyloid formation and toxicity by interacting with prefibrillar structures. *Faseb J.* **21,** 2312–2322.

Yoo, B. C., Kim, S. H., Cairns, N., Fountoulakis, M., and Lubec, G. (2001). Deranged expression of molecular chaperones in brains of patients with Alzheimer's disease. *Biochem. Biophys. Res. Commun.* **280,** 249–258.

Yoshizumi, M., Tsuchiya, K., and Tamaki, T. (2001). Signal transduction of reactive oxygen species and mitogen-activated protein kinases in cardiovascular disease. *J. Med. Invest.* **48,** 11–24.

Yu, B. P., and Chung, H. Y. (2006). Adaptive mechanisms to oxidative stress during aging. *Mech. Ageing Dev.* **127,** 436–443.

Yu, A. L., Fuchshofer, R., Kook, D., Kampik, A., Bloemendal, H., and Welge-Lüssen, U. (2009). Subtoxic oxidative stress induces senescence in retinal pigment epithelial cells via TGF-beta release. *Invest. Ophthalmol. Vis. Sci.* **50,** 926–935.

Zellweger, T., Kiyama, S., Chi, K., Miyake, H., Adomat, H., Skov, K., and Gleave, M. E. (2003). Overexpression of the cytoprotective protein clusterin decreases radiosensitivity in the human LNCaP prostate tumour model. *BJU Int.* **92,** 463–469.

Zhang, L. Y., Ying, W. T., Mao, Y. S., He, H. Z., Liu, Y., Wang, H. X., Liu, F., Wang, K., Zhang, D. C., Wang, Y., Wu, M., Qian, X. H., *et al.* (2003). Loss of clusterin both in serum and tissue correlates with the tumorigenesis of esophageal squamous cell carcinoma via proteomics approaches. *World J. Gastroenterol.* **9,** 650–654.

Zhang, H., Kim, J. K., Edwards, C. A., Xu, Z., Taichman, R., and Wang, C. Y. (2005). Clusterin inhibits apoptosis by interacting with activated Bax. *Nat. Cell Biol.* **7,** 909–915.

Zhou, W., Janulis, L., Park, I. I., and Lee, C. (2002). A novel anti-proliferative property of clusterin in prostate cancer cells. *Life Sci.* **72,** 11–21.

Zhu, X., Raina, A. K., Perry, G., and Smith, M. A. (2004). Alzheimer's disease: The two-hit hypothesis. *Lancet Neurol.* **3,** 219–226.

Zwain, I., and Amato, P. (2000). Clusterin protects granulosa cells from apoptotic cell death during follicular atresia. *Exp. Cell Res.* **257,** 101–110.