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Original Contribution

Differential effects of clusterin/apolipoprotein J on cellular growth and survival

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Abstract

The secreted clusterin/apolipoprotein J (CLU) protein form is a ubiquitously expressed heterodimeric glycoprotein which is differentially regulated in many severe physiological disturbance states including cell death, ageing, cancer progression, and various neurological diseases. Despite extensive efforts CLU function remains an enigma, the main cause being the intriguingly distinct and usually opposed functions in various cell types and tissues. In the current report we investigated the effects of CLU on cellular growth and survival in three human osteosarcoma (OS) cell lines, namely KH OS, Sa OS, and U-2 OS that express very low, moderate, and high endogenous steady-state CLU amounts, respectively. We found that exposure of these established OS cell lines or primary OS cells to genotoxic stress results in CLU gene induction at distinct levels that correlate negatively to CLU endogenous amounts. Following CLU-forced overexpression by means of an artificial transgene, we found that although extracellular CLU inhibits cell death in all three OS cell lines, intracellular CLU has different effects on cellular proliferation and survival in these cell lines. Transgenic KH OS cell lines adapted to moderate intracellular CLU levels were growth-retarded and became resistant to genotoxic and oxidative stress. In contrast, transgenic Sa OS and U2 OS cell lines adapted to high intracellular CLU amounts were sensitive to genotoxic and oxidative stress. In these two cell lines, the proapoptotic CLU function could be rescued by caspase inhibition. To monitor the immediate effects of heterologous CLU overexpression prior to cell adaptation, we performed transient transfections in all three OS cell lines. We found that induction of high intracellular CLU amounts increases spontaneous apoptosis in KH OS cells and reduces DNA synthesis in all three cell lines assayed. On the basis of these novel findings we propose that although extracellular CLU as well as intracellular CLU at low/moderate levels is cytoprotective, CLU may become highly cytostatic and/or cytotoxic if it accumulates intracellularly in high amounts either by direct synthesis or by uptake from the extracellular milieu.

Keywords: Apoptosis; Clusterin/apolipoprotein J; Genotoxic stress; Necrosis; Oxidative stress; Free radicals

Introduction

Human CLU[1] is a heterodimeric glycoprotein that was initially purified from serum [1] and identified as an apolipoprotein [2]. Following these studies CLU has been found in all human body fluids analyzed [3]. Not only does CLU function as an apolipoprotein but it is also implicated in additional intra- or extracellular processes. The CLU gene encodes for a 449 amino acid polypeptide where the first 22 amino acids represent the classical hydrophobic secretory signal sequence. Maturation of the primary translation molecule includes disulfide bonding and subsequent conversion to a high-mannose endoplasmic reticulum-associated form of ~60-kDa; extensive additional N-linked glycozylation and proteolytical cleavage in the trans-Golgi

Abbreviations: ANOVA, analysis of variance; CLU, clusterin/apolipoprotein J; DMEM, Dulbecco's modified Eagle's medium; DXR, doxorubicin (adriamycin); HDOs, human diploid osteoblasts; HSPs, heat shock proteins; OS, osteosarcoma; PBS, phosphate-buffered saline; PDs, population doublings; SD, standard deviation.

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compartments results in the mature secreted heterodimeric CLU protein form of ~70–80 kDa [4]. Another CLU protein form of ~55 kDa, designated as nuclear CLU (n-CLU^{55}) [5], has been described to originate in MCF-7 cells during apoptosis from a ~49-kDa primary protein form (c-CLU^{49}). This c-CLU^{49} protein form originates from an alternatively spliced CLU transcript [6].

CLU has been implicated in several diverse physiological functions [7,8]. It may also function as an extracellular chaperone that stabilizes stressed proteins in a folding-competent state [9,10]. CLU is differentially regulated in many severe physiological disturbance states including kidney degenerative diseases, several neurodegenerative conditions, tumorigenesis, inflammation, and cell death [4,8,11,12]; most of these diseases are characterized by significant oxidative injury. The role of CLU during cell death remains largely elusive due to intriguingly distinct and usually opposed functions in an array of various cell types and tissues [4] as well as at CLU$^{-/-}$ mice [13,14]. Specifically, CLU overexpression in various cell lines either could not confer any protection to apoptosis-inducing agents or functioned proapoptotically [5,13,15–17]. In contrast, use of antisense oligonucleotides targeting CLU resulted in significant chemosensitivity in prostate cancer [18], while CLU gene overexpression in human androgen-dependent LNCaP prostate cells rendered them highly resistant to androgen ablation in vivo [19] and to treatment with chemotherapeutic agents [20]. CLU function during oxidative stress is similarly obscure. For example, CLU exerts a proapoptotic function in a human epidermoid cancer cell line (A431 cells) treated with a photodynamic therapy that induces severe oxidative stress and apoptosis [17], but CLU protects the A431 cells, a porcine proximal tubular cell line (LLC-PK$_1$ cells), and normal human fibroblasts from various oxidative stresses including hydrogen peroxide, superoxide anion, hyperoxia, UVA exposure, ethanol, and *tert*-butylhydroperoxide [21–23]. We have cloned CLU as a senescence-induced gene [24] and found that it is also overexpressed in normal human diploid fibroblasts exposed to various types of stress [24,25] as well as during several diseases related to advanced aging and increased oxidation (e.g., atherosclerosis) [26]. Based on these contradictory findings, we recently proposed that CLU function might depend on the cellular context where it is expressed [4].

To address this hypothesis, we use as a model system three human OS cell lines (U-2 OS, Sa OS, and KH OS) that represent examples of a distinct genetic background, distinct endogenous CLU levels, and different CLU accumulation rates following cell exposure to various types of stress. These OS cell lines are sensitive to the cytotoxic and cytostatic effects induced by either genotoxic (e.g., DNA topoisomerase I, II inhibitors) or oxidative (e.g., H_2O_2) stress [27]. Using this model system we have recently shown that siRNA-mediated CLU knock down results in growth retardation, higher rates of spontaneous endogenous apoptosis, and significant cell sensitization to both genotoxic and oxidative stress-inducing agents [27]. In support, we observed similar effects in CLU knock down PC-3 prostate cancer cells [27].

In the current report we pursue our studies and analyze the biological effects of CLU forced overexpression in OS cells by means of an artificial transgene. To our surprise we found that transient high levels of CLU decrease DNA synthesis in all three OS cell lines and they enhance spontaneous apoptosis in KH OS cells. Following cell death induction by serum deprivation, genotoxic or oxidative stress, CLU either inhibits or promotes cell death depending on the site of action, the type of the apoptotic stimulus, and the cellular context. These novel data exemplify the chimerical CLU function in cell lines that originated from the same tissue and introduce for the first time the several parameters that affect CLU function during cell death.

Materials and methods

Cell lines and tissue culture

OS cell lines were purchased from American Tissue Culture Collection. Human diploid Osteoblasts (HDOs) were a kind offer of Dr. Rattan and were always subcultured at a split ratio 1:2 to maintain maximal growth. Osteo-1 to Osteo-4 cultures were established from biopsies derived from patients with primary osteosarcoma as previously described [28]; none of these patients had received any chemotherapy. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Life Technologies Inc.) supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, and 1% (v/v) nonessential amino acids (complete medium).

Transient or stable CLU overexpression in OS cell lines

The human CLU full-length cDNA was isolated from the pGAD10 vector (a kind offer of Dr. Howe) and subcloned directly into the pcDNA3.1/Myc-His$^+$ mammalian expression vector (Invitrogen Life Technologies Inc.). Cells were transfected with either the pcDNA3.1 empty vector or the pcDNA3.1^{CLU} construct by using the Lipofectamine 2000 reagent (Invitrogen) according to manufacturer's instructions. Transient CLU overexpression was assayed 24 to 48 h posttransfection. To develop cell lines that overexpress CLU stably, transfectants were selected in complete medium containing 400 μg/ml geneticin for 3 weeks. To avoid clonal variation we selected pools of transduced cells; each pool of stably transduced cells represented the outcome of a single transfection experiment. We developed four pools of transfected cells with the pcDNA3.1 empty vector/cell line and six pools using the pcDNA3.1^{CLU} construct. All transfectants were tested for CLU protein expression levels by immunoblotting analysis. The presented results refer to the mean observed after assaying at least two pcDNA3.1 and three pcDNA3.1^{CLU} distinct pools of transfected cells.