**Molecular Pathways**

**Targeting the Cytoprotective Chaperone, Clusterin, for Treatment of Advanced Cancer**

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**Abstract**

Many strategies used to kill cancer cells induce stress-responses that activate survival pathways to promote emergence of a treatment resistant phenotype. Secretory clusterin (sCLU) is a stress-activated cytoprotective chaperone up-regulated by many varied anticancer therapies to confer treatment resistance when overexpressed. sCLU levels are increased in several treatment recurrent cancers including castrate resistant prostate cancer, and therefore sCLU has become an attractive target in cancer therapy. sCLU is not druggable with small molecule inhibitors, therefore nucleotide-based strategies to inhibit sCLU at the RNA level are appealing. Preclinical studies have shown that antisense oligonucleotide (ASO) or siRNA knockdown of sCLU have preclinical activity in combination with hormone- and chemotherapy. Phase I and II clinical trial data indicate that the second generation ASO, custirsen (OGX-011), has biologic and clinical activity, suppressing sCLU expression in prostate cancer tissues by more than 90%. A randomized study comparing docetaxel-custirsen to docetaxel alone in men with castrate resistant prostate cancer reported improved survival by 7 months from 16.9 to 23.8 months. Strong preclinical and clinical proof-of-principle data provide rationale for further study of sCLU inhibitors in randomized phase III trials, which are planned to begin in 2010. *Clin Cancer Res; 16(4); 1088–93. ©2010 AACR.*

**Background**

Development of treatment resistance is a common feature of solid tumor malignancies and the underlying basis for most cancer deaths. Treatment resistance results from multiple, stepwise changes in DNA structure and gene expression-a Darwinian interplay of genetic and epigenetic factors arising, in part, from selective pressures of treatment. In most solid tumors this evolutionary process cannot be attributed to singular genetic events, involving instead many cumulative changes in gene structure and expression that facilitate cancer cell growth and survival. Examples include altered expression of genes regulating drug penetration, transport, and metabolism (1), or those regulating the apoptotic rheostat of cancer cells. In advanced prostate cancer, for example, treatment resistance is manifest by progression to castration resistant prostate cancer (CRPC) via mechanisms attributed to reactivation of androgen receptor axis (2), alternative mitogenic growth factor pathways (3–5), and stress-induced prosurvival gene (6–8) and cytoprotective chaperone networks (9, 10).

Molecular chaperones, including heat-shock proteins (Hsp), help cells cope with stress-induced protein misfolding, aggregation, and denaturation and play prominent roles in cellular signaling and transcriptional regulatory networks. Chaperones act as genetic buffers stabilizing the phenotype of various cells and organisms at times of environmental stress, enhancing the Darwinian fitness of cells during transformation, progression, and treatment resistance (11). Indeed, the heat shock response is a highly conserved protective mechanism for eukaryotic cells under stress, and is associated with oncogenic transformation, proliferation, survival, and thermotolerance (12). Thus, targeting molecular chaperones with multifunctional roles in endoplasmic reticular (ER) stress and cellular signaling and transcriptional regulatory networks associated with cancer progression and treatment resistance is an attractive and rational therapeutic strategy. Of special relevance to treatment-resistant cancers are those chaperones' up-regulated anticancer therapies that function to inhibit treatment-induced cell death, including clusterin (CLU; refs. 10, 13) and Hsp27 (9, 14). This review summarizes the roles of CLU in cancer cell survival and treatment resistance, and the preclinical pharmacology and early clinical trial results for custirsen (OGX-011), a second generation antisense inhibitor targeting CLU.

**CLU Structure and Function**

Secretory CLU (sCLU) is a multifunctional, stress-induced, ATP-independent molecular chaperone, previously known as apolipoprotein J, testosterone-repressed prostate message-2, ionizing radiation-induced protein-8, SP 40-40, complement lysis inhibitor, gp80, glycoprotein III, or sulfate glycoprotein-2. sCLU is expressed in most tissues and human fluids analyzed. sCLU is a versatile molecular chaperone containing amphipathic and coiled-coil helices...
in addition to large intrinsic disordered regions. These properties of sCLU resemble survival chaperones associated with tissue injury and pathology like acute phase protein haptoglobin (15) and small Hsps (16). Indeed, sCLU is involved in many biological processes ranging from mammalian and prostate gland involution to amyloidosis and neurodegenerative disease, as well as cancer progression and treatment resistance (17).

CLU is a single copy gene, organized into nine exons (eight introns) and a 5′-untranslated region, located on chromosome 8p21-p12 extending more than 16 kb (18). In humans, the CLU gene codes for two secretory isoforms (sCLU-1, sCLU-2), originating from transcriptional start sites in exons 1 and 2, respectively; only sCLU-2 is expressed in subprimates. sCLU is an ER-targeted, 449-amino acid polypeptide that represents the predominant translation product of the human gene (Fig. 1A). Proteolytic removal of the ER-targeting signal peptide produces a 60-kDa ER-associated, high mannose, cytoplasmic form (sCLUc; ref. 19). sCLUc is further glycosylated in the Golgi and cleaved at Arg227-Ser228 into two 40-kDa α- and β-subunits. These subunits are assembled in an antiparallel manner into an 80-kDa mature, secreted, heterodimeric form (sCLUs), in which its cysteine-rich centers are linked by five disulfide bridges and flanked by two coiled coil α-helices and three predicted amphipathic α-helices. The coiled-coil domain is a highly versatile protein folding and oligomerization motif, facilitating its interaction with client proteins involved in many protein signal-transducing events. Although sCLU is cytoprotective and anti-apoptotic, a pro-apoptotic activity ~55-kDa nuclear (nCLU) splice variant lacking exon II and the ER signal peptide has been described (20, 21).

Promoter sequences of CLU gene are conserved during evolution, and include stress-associated sites like activator-protein-1 (AP-1), AP-2, stimulatory element (SP-1), heat shock element (HSE), CAMP response element (CRE), and a "CLU-specific element" (CLE) recognized by HSF1-1/HSF2 heterocomplexes (22). Steroid response elements include glucocorticoid response element (GRE; refs. 23–25) and androgen response element (ARE) sites (26). CLU promoter regions contain CpG-rich methylation domains (23, 27), indicating CLU may be regulated by DNA methylation and histone acetylation (28); indeed, 5-aza-2′-deoxycytidine significantly increases sCLU expression in prostate cancer cells (29). Additionally, sCLU expression increases downstream of survival signaling pathways, including IGF-1 via Src-Mek-Erk-EGR-1 (30) and cytokines via Jak/STAT1 (Fig. 1B; ref. 31).

sCLU functions to protect cells from many varied therapeutic stressors that induce apoptosis, including androgen or estrogen withdrawal, radiation, cytotoxic chemotherapy, and biologic agents (32). Mechanisms by which sCLU exerts these effects are not fully elucidated. Because sCLU binds to a wide variety of biological ligands, and is regulated by HSF1 (22), an emerging view suggests that sCLU functions like small Hsps to chaperone and stabilize conformations of proteins at times of cell stress. Indeed, sCLU is more potent than other Hsps at inhibiting stress-induced protein precipitation. As a functional homolog of sHsps, CLU has chaperone activity with a potent ability to influence the amorphous and fibrillar aggregation of many different proteins. CLU inhibits stress-induced protein aggregation by binding to exposed regions of hydrophobicity on non-native proteins to form soluble, high molecular mass complexes (33, 34). During amorphous aggregation of proteins, sCLU interacts with slowly aggregating species on the off-folding pathway. Immunoaffinity depletion of CLU from human plasma renders proteins in this fluid more susceptible to aggregation and precipitation (35). sCLUs interacts with stressed cell surface proteins (e.g., receptors) to inhibit pro-apoptotic signal transduction (16). sCLU inhibits ER stress, retro-translocating from the ER to the cytosol to inhibit aggregation of intracellular proteins and prevent apoptosis (36). Interestingly, and likely related to its role in inhibiting protein aggregation, sCLU is the most abundant protein associated with β-amyloid deposits in Alzheimer’s disease (33). Collectively, the preceding indicates sCLU plays an important role in unfolded protein and ER stress responses.

Many reports also document that sCLUs inhibits mitochondrial apoptosis. For example, sCLUs suppresses p53-activating stress signals and stabilizes cytosolic Ku70-Bax protein complex to inhibit Bax activation (37). sCLU specifically interacts with conformationally altered Bax to inhibit apoptosis in response to chemotherapy drugs (38). sCLU knockdown alters the ratios of anti-apoptotic Bcl-2 family members, disrupting Ku70/Bax complexes and Bax activation (37, 38). In addition, sCLUs increases Akt phosphorylation levels and cell survival rates (39). sCLU induces epithelial-mesenchymal transformation by increasing Smad2/3 stability and enhancing TGF-β-mediated Smad transcriptional activity (40). sCLU also promotes prostate cancer cell survival by increasing NF-κB nuclear transactivation, acting as a ubiquitin-binding protein that enhances COMMD1 and IκB proteasomal degradation via interaction with E3 ligase family members (41). sCLU knockdown stabilized COMMD1 and IκB, suppressing NF-κB translocation to the nucleus, and suppressing NF-κB-regulated gene signatures (Fig. 1B).

Clinical-Translational Advances

CLU is expressed in many human cancers, including breast, lung, bladder, kidney, colon-rectum, and prostate (42–46). In prostate, sCLU was originally cloned as “testosterone-repressed prostate message 2” (TRPM-2; ref. 47) from regressing rat prostate, but was later defined as a stress-activated and apoptosis-associated, rather than an androgen-repressed, gene (10). sCLU levels increase following castration and in castrate resistant prostate cancer models (10, 13). In human prostate cancer, sCLU levels are low in low grade untreated hormone-naive tissues, but increase with higher Gleason score (46) and within weeks after androgen deprivation (48). sCLU expression correlates with loss of the tumor suppressor gene Nkx3.1 during the initial stages of prostate tumorigenesis in Nkx3.1 knockout mice (49). Interestingly, high levels of sCLU expression associate with migration, invasion, and metastasis, whereas sCLU...
Fig. 1. A, CLU structure: The cytoplasmic precursor peptide (sCLUc) is cleaved proteolytically between amino acids 22 and 23 to remove the signal peptide (turquoise) and between residues 227 and 228 to generate the α (green) and β (orange) chains. The α and β chains are assembled in antiparallel to form a mature heterodimer (sCLUs). The cysteine rich centers (red) are linked by five disulfide bridges (red ellipses). These are flanked by two predicted coiled-coil α-helices (blue) and three predicted amphipathic α-helices (purple). Six N-glycosylation sites are indicated as yellow dots. B, role of CLU in cancer progression. sCLU is up-regulated by stress-activated transcription factors (e.g., HSF-1), with ER stress, and downstream of cytokines (via JAK/stat) and IGF-1R (via Src-MEK-ERK-Erg-1) signaling pathways. Once up-regulated, CLU exerts a feed-forward loop involving ERK activation, which leads to GSK-3β phosphorylation and Slug activation. Up-regulation of CLU increases p-AKT levels, facilitating downstream cascades that include NF-κB. CLU increases cell survival through mechanisms involving inhibition of ER stress, suppressing Bax activation with mitochondrial sequestration of cytochrome C, and NF-κB nuclear translocation by enhancing proteasomal degradation of IκB and COMMD1.
silencing induces mesenchymal-epithelial-transition via inhibition of Slug (50). Experimental and clinical studies associate sCLU with development treatment resistance, in which sCLU suppresses treatment-induced cell death in response to androgen withdrawal, chemotherapy, or radiation (10, 13, 48). Overexpression of sCLU in human prostate LNCaP cells accelerates progression after hormone or chemotherapy (13), identifying sCLU as a anti-apoptotic gene up-regulated by treatment stress that confers therapeutic resistance when overexpressed.

sCLU is not a traditional druggable target and can only be targeted at mRNA levels. An antisense inhibitor targeting the translation initiation site of human exon II CLU (OGX-011) was developed at the University of British Columbia and out-licensed to OncoGenex Pharmaceuticals Inc. OGX-011, or custirsen, is a second-generation antisense oligonucleotide with a long tissue half-life of ∼7 days, which potently suppresses sCLU levels in vitro and in vivo. OGX-011 improved the efficacy of chemotherapy, radiation, and hormone withdrawal by inhibiting expression of sCLU and enhancing apoptotic rates in preclinical xenograft models of prostate, lung, renal cell, breast, and other cancers (51–53).

To date, more than 300 patients have been treated with custirsen in six phase I and II clinical trials. The first-in-human phase I study with custirsen used a novel neoadjuvant design to identify effective biologic dosing of custirsen to inhibit sCLU expression in human cancer (54). In this dose-escalation study, cohorts of three to six patients with localized prostate carcinoma and high risk features were treated with custirsen in doses of up to 640 mg given as a 2-hour intravenous infusion on days 1, 3, 5, 8, 15, 22, and 29 with prostatectomy done within 7 days of the last OGX-011 dose. Neoadjuvant androgen deprivation was administered concurrently. The presurgical design was used to correlate changes in expression of sCLU to drug dose received and drug levels within the prostate tissue itself. In this study, treatment was well tolerated and at doses of 320 mg and higher, concentrations of full-length custirsen were achieved that were associated with preclinical activity. Mean tissue concentrations increased in a dose-dependent manner to 4.82 μg/g of prostate tissue at a 640-mg dose, corresponding to a concentration of nM. Custirsen produced statistically significant, dose-dependent >90% knockdown of sCLU in normal and tumor tissue. Furthermore, mean apoptotic indices increased from 7.1 to 21.2%. Thus, clinical pharmacodynamic data clearly indicate that custirsen is biologically active in humans and identified 640 mg as the optimal biologic dose for phase II trials.

Plasma pharmacokinetic parameters have been similar across phase I studies including when custirsen was combined with chemotherapy and decreases in serum sCLU have been consistently observed (55, 56). A phase II trial of 85 patients with non-small cell lung cancer (NSCLC) treated with combined custirsen and gemcitabine-cisplatin chemotherapy (56) reported an objective response rate of 23% and median overall survival of 383 days with 58% surviving >1 year. This overall survival data were considered clinically significant as compared with prior clinical trials data with chemotherapy alone, justifying further studies in NSCLC.

Randomized phase II studies were conducted in patients with CRPC because of limitations in interpreting antitumor responses of novel biologics when combined with chemotherapy (57). 81 patients with chemo-naïve, metastatic CRPC were randomized to receive either docetaxel-custirsen or docetaxel-alone. The median cycles delivered for docetaxel-custirsen was nine compared with seven for docetaxel-alone. There was evidence of biologic effect with 18% decrease in mean serum sCLU in patients treated with docetaxel-custirsen versus 8% increase in controls (P = 0.0005). Median progression free survival was 7.3 months for docetaxel-custirsen and 6.1 months for docetaxel-alone arm. Median overall survival on the docetaxel-custirsen arm was 23.8 months, ~7 months longer than those receiving docetaxel-alone (16.9 months). Multivariate analysis of factors associated with improved overall survival identified Eastern Cooperative Oncology Group (ECOG) performance status of 0 [hazard ratio (HR) = 0.28, P < 0.0001], and treatment assignment to the docetaxel-custirsen treatment arm (HR = 0.49, P = 0.012). Given the survival outcomes observed in the docetaxel-custirsen arm, further evaluation of the combination is warranted in comparative randomized phase III studies.

Another trial of docetaxel-recurrent CRPC randomized 42 patients to receive either docetaxel or mitoxantrone both combined with custirsen, thus evaluating the hypotheses that custirsen could reverse docetaxel resistance or improve mitoxantrone efficacy in a chemo-resistant population (58). PSA declines of ≥30% were seen in 55% of docetaxel-custirsen patients and 32% of mitoxantrone-custirsen patients. Pain responses were also seen in >50% of patients and after a median follow-up of 13.3 months, 60% of patients were alive in both arms. These results are also of interest considering PSA response rates of <20% and median survival <12 months is usually reported in patients with docetaxel-resistant CRPC receiving second-line chemotherapy (59), supporting further studies for second line indications for CRPC.

In summary, sCLU is a stress-activated cytoprotective chaperone that confers broad-spectrum treatment resistance when overexpressed. Preclinical and randomized clinical data with custirsen confirm potent suppression of sCLU expression and prolonged overall survival in CRPC. Further studies of custirsen in randomized phase III trials are justified and are due to begin in 2010.

Disclosure of Potential Conflicts of Interest

M. Gleave, consultant, OncoGenex Technologies, Sanofi-Aventis, AstraZeneca; commercial research support, OncoGenex Technologies, Sanofi-Aventis, Astra-Zeneca, Pfizer; ownership interest, OncoGenex Technologies. K. Chi, consultant, Sanofi-Aventis, Astra-Zeneca; commercial support, Astra-Zeneca. The other author declared no conflicts of interest.

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