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Clusterin and Chemoresistance

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Abstract

Resistance to anticancer agents is one of the primary impediments to effective cancer therapy. Chemoresistance occurs not only to clinically established therapeutic agents but also to novel targeted therapeutics. Both intrinsic and acquired mechanisms have been implicated in drug resistance but it remains controversial which mechanisms are responsible that lead to failure of therapy in cancer patients. Recent focus has turned to clusterin (CLU) as a key contributor to chemoresistance to anticancer agents. Its role has been documented in prostate cancer for paclitaxel/docetaxel resistance as well as in renal, breast, and lung tumor cells. Moreover, it is abnormally upregulated in numerous advanced stage and metastatic cancers spanning prostate, renal, bladder, breast, head and neck, colon, cervical, pancreatic, lung carcinomas, melanoma, and lymphoma. It is noteworthy that only the cytoplasmic/secretory clusterin form (sCLU), and not the nuclear form, is expressed in aggressive late stage tumors, which is in line with its antiapoptotic function. Most significantly, sCLU expression is documented to lead to broad-based resistance to other unrelated chemotherapeutic agents such as doxorubicin, cisplatin, etoposide, and camphothecin. Resistance to targeted death-inducing molecules, tumor necrosis factor, Fas and TRAIL, or histone deacetylase inhibitors can also be mediated by sCLU. Expression of sCLU may be an adaptive response to genotoxic and oxidative stresses but this adaptive response could pose a threat in malignant cells being treated with cytotoxic agents by enhancing their survival potential. The actual mechanisms for sCLU induction are unclear but STAT1 is required for its constitutive upregulation in docetaxel-resistant tumor cells. Known as a protein chaperone, sCLU appears to stabilize Ku70/Bax complexes, sequestering Bax from its ability to induce mitochondrial release of cytochrome *c* that triggers cell apoptosis. Thus, sCLU has a key role in preventing apoptosis induced by cytotoxic agents and has the potential to be targeted for cancer therapy.

I. INTRODUCTION

Cancer is a daunting disease to cure especially when it is diagnosed at an advanced stage that has a high risk of progression to metastasis. Chemoresistance to both standard anticancer agents and novel targeted therapeutics is a key barrier and remains one of the most pressing issues as the disseminated tumor becomes refractory to the drug, eventually failing all clinically proven drugs available for the tumor type (Borst *et al.*, 2007). Understanding the mechanisms of resistance may therefore lead to improved cancer therapeutics. Intrinsic pathways already existent within the tumor cell may participate in resisting cell death by cytotoxic drugs, yet other new pathways triggered during drug treatment can also play a role in preventing cell death. Despite intense effort to unravel the intrinsic and extrinsic pathways that mediate chemoresistance, it is still unclear which specific process is dominant in tumor cell survival. Clusterin (CLU), in its cytoplasmic secretory form (sCLU), has the unique property in mediating chemoresistance to numerous unrelated anticancer agents and its presence has been observed in a variety of solid tumors and lymphoma (Trougakos *et al.*, 2009a). In this chapter, evidence will be provided that elucidates the role of sCLU in chemoresistance and the potential of targeting sCLU to overcome drug resistance in the clinic will be discussed.

II. ASSOCIATION OF sCLU WITH CHEMORESISTANCE

For almost a decade since its discovery, CLU was primarily considered a marker of cell death because of its appearance initially described in castration-induced programmed cell death in the normal rat prostate (Bettuzzi *et al.*, 1989; Leger *et al.*, 1987) and later in other organ systems undergoing massive apoptosis. For example, CLU expression is induced in renal tubule tissue damaged by ligation, embryonic fetal cells in regressing interdigital tissue in the forming forelimbs, and murine bladder tumors undergoing cytotoxic death during cyclophosphamide treatment (Buttyan *et al.*, 1989). However, this view was overturned when a key set of experiments analyzing cell death induced by tumor necrosis factor (TNF) in LNCAP human prostate tumor cells provided the first report that CLU may actually be cytoprotective (Sensibar *et al.*, 1995). The evidence came from the following observations. Upon TNF treatment, CLU did rise but declined prior to observation of cell death. More significantly, transfection with antisense CLU to deplete it in LNCAP tumor cells resulted in increased apoptosis and the reverse was seen with CLU overexpression, which endowed the tumor cells with the ability to survive better and resist the cytotoxic effect of TNF (Sensibar *et al.*, 1995). Thus, CLU plays a critical role in protection against TNF-induced cell death. This seminal finding was reproduced in another human prostate tumor cell line, PC3, which constitutively expressed more sCLU than LNCAP and defined the linkage between sCLU and resistance to TNF-induced apoptosis (Sintich *et al.*, 1999). Clearly, purified sCLU added to LNCAP resulted in its ability to resist TNF cytotoxicity while pretreatment of PC3 tumor cells with anti-CLU antibodies sensitized them to TNF-mediated death. Thus, extracellular sCLU is responsible for the protective effects against TNF.

In addition to TNF resistance, androgen independence can also be attributed to sCLU. Prostate cancer is initially treated with surgery or irradiation for localized disease but long-term disease control can only be achieved with hormonal therapy that suppress androgen receptor signaling (Loblaw *et al.*, 2007; Taplin, 2007). Prostate tumor cells are exquisitely dependent on androgen and its receptor for growth signaling, but therapies directed against this pathway inevitably fail as resistance occurs (Tammela, 2004). In order to understand this phase of progression in prostate cancer, LNCAP human prostate tumor cells, which are androgen-dependent, were employed to investigate the participation of sCLU. LNCAP, which does not constitutively express sCLU, is normally highly sensitive to apoptosis upon androgen withdrawal from the culture medium. However, when stably transfected with the CLU gene, these tumor cells were found to gain the ability to survive and resist androgen ablation (Miyake *et al.*, 2000c). In another androgen-dependent tumor model, Shionogi murine tumors in male mice usually undergo complete regression upon castration but recurrence is common after a month with accompanying androgen-independence. These recurrent tumor cells were found to express sCLU in both its cytoplasmic 60 kDa form and secretory heterodimeric 40 kDa forms which are those established to be critical for cell survival (Miyake *et al.*, 2000c). *In vivo* administration of antisense CLU oligonucleotides into Shionogi tumor-bearing mice was demonstrated to significantly accelerate tumor regression and substantially delayed the development of androgen-independent tumors. These findings indicate that sCLU is instrumental in acting as an antiapoptotic agent and facilitates survival and growth of tumors that no longer require androgen for their maintenance.

Using these two tumor cell lines, sCLU was also implicated in the development of chemoresistance to paclitaxel (Miyake *et al.*, 2000b). Once androgen independence gains a foothold in prostate tumor cells, the drug of choice is proven to be the taxanes, including paclitaxel (taxol) and docetaxel (Petrylak *et al.*, 2004; Tannock *et al.*, 2004). This is because androgen-independence is associated with the induction of bcl-2, an antiapoptotic protein (McDonnell *et al.*, 1992). Although the taxanes primarily work through microtubule

disruption, they also are highly effective in disrupting bcl-2 phosphorylation, required for its antiapoptotic function (Haldar *et al.*, 1997; Scatena *et al.*, 1998). This property led to the clinical use of taxanes, particularly, docetaxel, in the treatment of advanced refractory prostate cancer (Petrylak *et al.*, 2004; Tannock *et al.*, 2004). Nevertheless, it soon became obvious that resistance to paclitaxel or docetaxel can often occur, leading to treatment failure and the spread of metastasis, particularly to bone (Galletti *et al.*, 2007). LNCAP is highly sensitive to paclitaxel *in vitro*, but upon transfection with sCLU, it was found to withstand such treatment and resisted apoptotic cell death. *In vivo* in nude mice, parental human LNCAP tumors readily regressed upon castration and administration of paclitaxel, but sCLU-overexpressing LNCAP survived such treatment. Data complementing these observations were also obtained in the sCLU-positive Shionogi tumors. Administration of antisense CLU alone did not cause tumor regression in mice bearing syngeneic Shionogi tumors, but this treatment together with paclitaxel was highly effective. Thus, the conclusion can be reached that sCLU overexpression helps to create a chemoresistant phenotype and sCLU ablation via specific antisense oligonucleotides may be required to chemosensitize resistant tumors to paclitaxel in hormone refractory prostate tumors. To prove this concept, androgen-independent PC3 prostate tumors were tested *in vitro* and *in vivo* in nude mice for susceptibility to paclitaxel. Apparently, PC3 tumor cells naturally express sCLU and it was confirmed that blockade of CLU via specific siRNA was first needed before these tumors could respond to paclitaxel to show shrinkage (Miyake *et al.*, 2000a). This finding with PC3 tumor cells was also reproduced by others (Trougakos *et al.*, 2004). The same property of CLU in chemoresistance to paclitaxel was observed in other tumor types, including renal, breast, and lung carcinoma. Pretreatment of Caki-2 human renal carcinoma cells with antisense-CLU greatly enhanced chemosensitivity to paclitaxel *in vitro* and *in vivo* in nude mice (Zellweger *et al.*, 2001). Using another model of breast cancer where taxanes are the established choice for management of metastatic disease, antisense-CLU effectively chemosensitized MCF7 and MD-MB231 breast tumor cells to paclitaxel-induced apoptosis (So *et al.*, 2005). Such results were also obtained with human A549 lung carcinoma cells responding to paclitaxel (July *et al.*, 2004). Thus, the potential of targeting sCLU in sensitizing tumor cells to chemotherapy has become an attractive new modality for cancer treatment.

III. ASSOCIATION OF sCLU WITH MULTIDRUG RESISTANCE

To further investigate the extent of chemoresistance conferred by sCLU, investigators began to explore other commonly used chemotherapeutic agents for cancer treatment. It was quickly found that cisplatin sensitivity could be modulated by sCLU. Cisplatin and its derivatives have a broad range of activity in malignant disease, covering testicular, ovarian, small cell and nonsmall cell lung, cervical, head and neck, colorectal and bladder cancers (Martin *et al.*, 2008). They work by binding to DNA and forming DNA adducts leading to intrastand or interstand cross-links, thus disrupting normal DNA structure and impairing proper DNA replication resulting in cell death (Rabik and Dolan, 2007). The development of cisplatin resistance is an unavoidable threat and several important modes of resistance have been uncovered, based on DNA repair enzymes (Martin *et al.*, 2008). In addition to these DNA repair mechanisms, however, resistance by other processes can also be a potent deterrent to cytotoxic death by cisplatin. In examining bladder cancer, it was found that antisense-CLU could enhance chemosensitivity to cisplatin in KoTCC-1 human bladder tumor cells *in vitro* (Miyake *et al.*, 2001a). As in PC3 prostate tumor cells, antisense CLU alone did not affect KoTCC-1 survival or proliferation *in vitro*. However, antisense CLU plus cisplatin treatment clearly suppressed tumor cell growth via induction of apoptosis. This method of CLU blockade was also effective *in vivo*, as systemic administration of CLU-specific antisense oligonucleotides greatly enhanced cisplatin sensitivity of KoTCC-1 in nude mice as compared to control oligonucleotides, leading to retardation in tumor

growth. A similar strategy used in Caki-l renal carcinoma cells confirmed that antisense-CLU can provide chemosensitization against cisplatin (Lee *et al.*, 2002). In another detailed analysis of SKOV3 ovarian tumor cells, it was definitely demonstrated that transfection with the nuclear form of CLU induced apoptosis while transfection with the sCLU form in the same ovarian tumor cells promoted survival against cisplatin, thus leaving no doubt as to the dual forms and functions of CLU (Wei *et al.*, 2009).

It became clear then that CLU could mediate multidrug resistance to a broad range of unrelated chemotherapeutic agents. For example, CLU overexpression in Mel-Juso melanoma cells was associated with an increase in drug resistance not only to paclitaxel but also to cisplatin and 5 fluorouracil *in vitro* (Hoeller *et al.*, 2005). In addition, 5182 melanoma cells that constitutively express sCLU, grew progressively in nude mice but this growth could be stemmed by *in vivo* treatment with antisense-CLU which allowed for chemosensitivity to dacarbazine-induced apoptosis and improved tumor responses (Hoeller *et al.*, 2005). Similar use of antisense CLU also raised sensitivity to gemcitabine in human bladder koTCC-1 tumor cells *in vitro* and *in vivo* (Miyake *et al.*, 2004a). Moreover, human fibrosarcoma cells transfected with sCLU were reportedly resistant to etoposide as well as campthothecin (Zhang *et al.*, 2005). In another study, in order to analyze chemoresistance, doxorubicin-resistant human osteosarcoma cells were developed by culture in increasing levels of the drug (Lourda *et al.*, 2007). These resistant tumor cells were found to show significantly less cell death normally induced by paclitaxel, cisplatin, or camphotecin. In yet another study, it was shown that DU145 and PC3 human prostate tumor cells already cultivated in docetaxel to develop resistance, were also resistant to TRAIL-induced cell death (Sallman *et al.*, 2007). This acquisition to TRAIL resistance was due to expression of sCLU, as shown by restoration of TRAIL sensitivity upon knockdown of CLU gene expression by specific siRNA. It can thus be concluded that multidrug resistance to a wide array of therapeutic agents used for management of cancer can be achieved by upregulation of sCLU in tumor cells.

IV. ASSOCIATION OF sCLU WITH RESISTANCE TO IRRADIATION AND OXIDATIVE STRESS

It has become apparent that CLU may also be protective against radiation therapy or oxidative stress. In androgen-dependent LNCAP tumor cells, the overexpression of CLU renders them significantly less sensitive to irradiation *in vitro*, as compared to nontransfected parental LNCAP tumor cells (Zellweger *et al.*, 2002). On the other hand, antisense CLU-specific oligonucleotides can reduce the expression of CLU in androgen-independent PC3 tumor cells and sensitize them to radiation-induced cell death. Radiation treatment of antisense-CLU transfected PC3 tumor cells induced a higher rate of apoptosis than the same treatment in mismatch control-transfected PC3. Thus, CLU can act as a cell survival protein that mediates radioresistance by prevention of apoptosis. In addition, CLU may also participate in resistance to oxidative stress. Oxidative stress is a major factor associated with the progression of prostate cancer via accumulation of DNA damage. It was shown that transfection of CLU into LNCAP tumor cells can suppress hydrogen peroxide (H_2O_2)-induced apoptosis (Miyake *et al.*, 2004b). This protection against oxidative stress was also mirrored in human osteosarcoma cells (Trougakos *et al.*, 2004). U-2OS osteosarcoma cells constitutively express high levels of sCLU and show resistance to both H_2O_2 and doxorubicin. Upon antisense-CLU transfection, these cells now gain sensitivity to both oxidative and genotoxic stress. Conversely, osteosarcoma cells that have low CLU expression, when cultured in doxorubicin to develop drug-resistant cell lines, acquire resistance to H_2O_2 and both types of resistance in the same cells was mediated by the upregulation of sCLU (Lourda *et al.*, 2007). It is pertinent that human diploid fibroblasts,

transfected with sCLU, can resist H_2O_2- and ethanol-mediated stress-induced premature senescence (Dumont *et al.*, 2002).

V. ASSOCIATION OF sCLU WITH PROGRESSIVE TUMORS

From the above findings, it stands to reason that, in cancer patients, tumors expressing sCLU are likely to display more aggressive behavior and respond less well to chemotherapy or radiation therapy. To address whether CLU is spontaneously upregulated in cancer, a wide distribution of human tumor biopsies were analyzed by numerous laboratories. One of the earliest observations of CLU overexpression was made in human gliomas where analysis of differential gene display between benign and malignant tissues identified a markedly increased level of CLU mRNA expression in astrocytomas and glioblastomas (Danik *et al.*, 1991). This was confirmed by *in situ* hybridization to detect CLU mRNA in the tissues. As a control, endothelial cells in the vasculature were analyzed and were found to be CLU negative. In renal carcinoma, tumor tissues were reported to contain three-fold more CLU-specific mRNA than the adjacent normal tissue (Parczyk *et al.*, 1994). Following these observations, a number of other tumor types were analyzed. With the availability of antibodies against CLU, subsequent reports also focused on CLU protein expression and its localization within the cell. In immunohistochemical analysis of CLU protein in 40 human prostate tumor specimens, it became clear that CLU is steadily increased as the grade of tumor rises, reflected by the Gleason Score, and it is restricted to the cytoplasm with little presence in the nucleus (Steinberg *et al.*, 1997). Normal prostate tissue had no CLU staining while benign hyperplastic tissue from the prostate showed a weak sCLU staining. Its staining intensified with higher forms of malignancy. This provided the first indication that protection from apoptosis by CLU may account in part for biologically aggressive behavior. Others have since confirmed that sCLU is present in prostate tumor tissues from radical prostatectomy of cancer patients and its level is even higher in androgen-independent tumors in a sample size of 128 (July *et al.*, 2002). Another study with a sample size of 172 prostate tumor archival material also showed that sCLU correlates with Gleason Score (Miyake *et al.*, 2006).

Similar observations were repeated in a comprehensive study of a large set of human breast cancer specimens, including 34 benign, 8 atypical hyperplastic, 18 *in situ* carcinoma, 54 invasive carcinoma, and 8 metastatic breast archival specimens (Redondo *et al.*, 2000). Analysis of 40 nonneoplastic glandular epithelia was included as a control and none of them expressed CLU. On the other hand, CLU was upregulated primarily in the cytoplasm and was closely associated with a corresponding increase in tumor progression, developing from normal tissue toward premalignant and advancing onto the malignant phenotype. In fact, highest CLU expression was in the lymph node metastasis, thus linking sCLU as a phenotypic determinant of the aggressive nature of breast cancer. This early work was confirmed by another report in a breast tumor tissue microarray that represents 379 samples (So *et al.*, 2005).

Upregulation of sCLU or its mRNA is now reported in tumor specimens representing ovarian (Hough *et al.*, 2001; Xie *et al.*, 2005), renal (Zellweger *et al.*, 2001), colon (Pucci *et al.*, 2004), lung (July *et al.*, 2004), melanoma (Hoeller *et al.*, 2005), pancreas (Mourra *et al.*, 2007), and cervical cancer (Watari *et al.*, 2008). In the colon, it is clearly demonstrated the transition of CLU detection from the nucleus to the cytoplasm reflects a growing aggression of the malignancy (Pucci *et al.*, 2004). It has thus become a universal observation that sCLU can be detected in most solid tumors and its level, particularly in the cytoplasm, corresponds with progressing stages of the disease. It is interesting that sCLU is absent in most leukemias and lymphomas, and is only detected in anaplastic large cell lymphoma (Wellmann *et al.*, 2000). Analysis of 198 well-characterized lymphomas, including T cell, B

cell, Hodgkin lymphomas, as well as 31 established leukemia/lymphoma cell lines demonstrated that only one category of lymphoma, anaplastic large cell lymphoma, overexpressed the CLU gene and immunostaining localized it to the cytoplasm.

VI. ASSOCIATION OF sCLU WITH RESISTANCE TO TARGETED THERAPY (TNF, FAS, TRAIL, HDAC INHIBITORS, HERCEPTIN)

In addition to drug resistance to clinically relevant chemotherapeutic agents and ionizing radiation, sCLU also has the power to protect against reagents that specifically target molecules involved in cell death. A set of TNF-related proteins released or expressed by both immune and nonimmune cells, such as TNF, FAS, and TRAIL, has the unique ability to trigger cell death by binding its specific receptors on target cells and inducing a common signal pathway that leads to caspase activation and apoptosis (Papenfuss *et al.*, 2008). This property has led to the pursuit of these molecules as anticancer agents. However, sCLU has the potency to block the function of these death receptors. In fact, the antiapoptotic function of sCLU was actually first uncovered by the analysis of TNF-induced cell death in LNCAP prostate tumor cells, as discussed in the beginning of this chapter (Sensibar *et al.*, 1995). This original observation suggested that CLU might also protect against other death-inducing molecules of the same family. Thus, another study took up Fas as the targeting agent and reported that overexpression of CLU in a human renal carcinoma cell line, ACHN, indeed, prevented apoptosis that is normally achieved with Fas ligation through anti-Fas antibody (Miyake *et al.*, 2001b). In addition, TRAIL resistance in human DU145 and PC3 tumor cells was traced to sCLU expression (Sallman *et al.*, 2007).

Another surface receptor whose targeting has produced remarkable clinical responses is the Her2 growth factor receptor. This receptor is displayed at an abnormally high level in various cancers including breast and ovary and Herceptin/Trastuzumab which is a specific humanized monoclonal antibody targeting the Her2 receptor is highly effective in treatment of cancers expressing this receptor (Nahta *et al.*, 2006). However, resistance also develops against it. One study has indicated that use of antisense CLU prior to treatment with Herceptin can enhance the sensitivity to this drug, suggesting that CLU may also play a negative role in this targeted therapy (Biroccio *et al.*, 2005). In addition, histone deacetylase (HDAC) inhibitors are the new generation chemotherapeutic agents that modify DNA-related gene transcription, and are proving to have efficacy against a variety of cancers (Kelly and Marks, 2005). However, CLU can interfere with its efficacy. Human breast tumor cells, MDA-MB231 and MDA-MB468, which were highly resistant to HDAC inhibitors, upon transfection with siRNA-CLU, became sensitive to cell death induced by TSA, a potent HDAC inhibitor (Liu *et al.*, 2009).

Overall, it is now evident that sCLU is a powerful mediator of cell survival that can block the effects of almost all known therapeutic agents. Conversely, its suppression can sensitize tumor cells against these reagents. Thus, it is important to elucidate the mechanism of its induction for expression.

VII. INDUCTION BY GENOTOXIC AND OXIDATIVE STRESSES

One of the earliest studies on TNF-induced cell death provided the first demonstration that TNF induces it, not to induce apoptosis as was widely believed at that time, but to prevent cell death as a cytoprotective reaction against a toxic insult (Sensibar *et al.*, 1995). This seminal report led to numerous reassessments of tumor cells responding to various cytotoxic agents, and it was confirmed that all toxic agents triggered the expression of CLU. Because CLU was first discovered as a survival protein in prostate tumor cells, a push was made to investigate if androgen ablation, irradiation, and paclitaxel treatment which are common

strategies for treatment of this cancer, upregulated CLU. All of these modalities were found to readily induce sCLU expression in human prostate tumor cells. In terms of hormonal ablation, androgen ablation linkage with CLU induction in prostate tumor cell lines (Cochrane *et al.*, 2007) were corroborated by analysis of human prostate tumor specimens taken from patients after hormonal therapy in comparison to those without treatment (Gleave *et al.*, 2001). More significantly, needle biopsies of prostate tumors obtained prior to neoadjuvant hormonal therapy were compared to the radical prostectomy specimens from the same patients after varying lengths of treatment (July *et al.*, 2002). It was clearly shown that the levels of sCLU in the treated samples were markedly higher than those before treatment, thus suggesting that sCLU expression is an adaptive response to provide cytoprotection against the anticancer regimen. In addition to androgen withdrawal in prostate cancer, estrogen withdrawal or paclitaxel treatment in breast tumor cells was also found to induce sCLU (So *et al.*, 2005). Such a response was also elicited by docetaxel treatment of prostate tumor cells (Patterson *et al.*, 2006). Irradiation had the same effect as reported in both prostate and breast tumor cells (Criswell *et al.*, 2003; Zellweger *et al.*, 2002). Other chemotherapeutic agents, such as cisplatin (Lee *et al.*, 2002; Miyake *et al.*, 2001a), doxorubicin (Lourda *et al.*, 2007; Trougakos *et al.*, 2004) as well as specific targeting agents such as Herceptin (Biroccio *et al.*, 2005) and HDAC inhibitors (Liu *et al.*, 2009; Ranney *et al.*, 2007) were also identified to be capable of inducing sCLU. Lastly, sCLU is also a responsive gene to oxidative stress, under H_2O_2 or ethanol treatment (Trougakos *et al.*, 2004). What is becoming apparent, then, is that sCLU is an adaptive response to not only genotoxic and cytotoxic stress but also oxidative stress. It is thus a unique survival protein that is called upon within a cell to withstand any damaging insult.

VIII. MECHANISM OF INDUCTION OF AND CYTOPROTECTION BY CLU

Given that the true biological nature of CLU is beginning to be better understood and the assignment of nCLU for proapoptotic function and sCLU for prosurvival function has finally gained consensus among the vested investigators (Trougakos *et al.*, 2009a), the molecular mechanisms for its production and function are still relatively unknown. In terms of its induction, its gene has been cloned and analysis of its promoter region has revealed several transcription regulators reported to be involved in gene transcription. Transcription factors that have been shown to interact with the CLU promoter and regulate its function include Egr-1 (Criswell *et al.*, 2005), AP-1 (Jin and Howe, 1997, 1999), Heat Shock Factor 1/2 (Loison *et al.*, 2006), Cdx1 (Suh *et al.*, 2001), B-MYB (Cervellera *et al.*, 2000), and c-MYC (Thomas-Tikhonenko *et al.*, 2004). In terms of STAT-related transcription factors, STAT1 but not STAT3 is identified to be involved in CLU gene expression (Patterson *et al.*, 2006). In addition, p53 and Nkx3.1 tumor suppressor genes appear to negatively regulate CLU gene expression (Criswell *et al.*, 2003; Song *et al.*, 2009) as does the b-catenin/Wnt pathway (Schepeler *et al.*, 2007).

How does sCLU mediate its cytoprotective function? It is apparently linked to its ability to bind Ku70/Bax complexes. A seminal report demonstrated that sCLU specifically binds activated Bax sequestering it from translocation to the mitochondria to induce cytochrome *c* release and apoptosis (Zhang *et al.*, 2005). Others have corroborated this finding and demonstrated that sCLU binds and stabilizes the Ku70/Bax complex in the cytoplasm, retaining it as a complex and preventing its release (Pucci *et al.*, 2009; Trougakos *et al.*, 2009b). Thus, it is of significance that its mechanism of action is linked to the Bcl-2 family of proteins that are potent in controlling the fate of a cell. In this case, sCLU clearly associated with a specific member, Bax. It is also of interest that the mechanism of action of nCLU is to bind the Ku70/Bax complex (Sawada *et al.*, 2003; Yang *et al.*, 2000). Although Ku70 is established to bind Bax in the cytoplasm to prevent its activation (Sawada *et al.*, 2003), it is also found in the nucleus where Ku70 was originally discovered as a critical

component of the DNA repair machinery (Wang *et al.*, 1998). Thus, the proapoptotic function of nCLU might be to sequester, in this case, Ku70, which is critical for repair in DNA double-strand breaks in the nucleus. It is intriguing that sCLU works in the cytoplasm by stabilizing Bax/Ku70 to sequester Bax from inducing apoptosis, while nCLU works in the nucleus by stabilizing Bax/Ku70 to sequester Ku70 from its DNA repair function.

Another pathway by which sCLU might act is via NF-κB. Being a protein stabilizer, sCLU apparently can also stabilize iKbα, thus preventing its degradation which is needed to release the p50/p65 NF-κB heterodimer for entry into the nucleus to act as a transcription factor (Santilli *et al.*, 2003; Takase *et al.*, 2008). Another means by which sCLU can affect cell survival is via its receptor. Interestingly, sCLU has been shown to bind its cell surface receptor, megalin, in a rat prostate cell line and induce AKTactivation which then can phosphorylate Bad, causing a decrease in cytochrome *c* release, thus favoring cell survival (Ammar and Closset, 2008).

IX. STRATEGIES TO BLOCKADE CLU FOR CHEMOSENSITIZATION IN CANCER CELLS

It is clear that resistance to anticancer drugs is a major obstacle in the cure of cancer patients. Multidrug resistance often develops against clinically useful chemotherapeutics and it is also becoming evident that resistance against newer targeted therapeutics can occur. A wide spectrum of intrinsic and extrinsic mechanisms has been proposed for the development of multidrug resistance but it is difficult and time-consuming to attack each mechanism to prevent drug resistance. The development of strategies to circumvent drug resistance poses a frustrating challenge. The emerging realization that sCLU is common to many advanced cancer types and that it can mount resistance to a large array of chemotherapeutic compounds with unrelated mechanisms has brought the impetus to target sCLU to treat multidrug resistance. Antisense technology has facilitated such targeting in the clinic. A second generation phosphorothioate antisense oligonucleotide complementary to the CLU mRNA translation initiation site has been developed which showed high efficiency *in vitro* in blocking CLU expression and in chemosensitization to several drugs, including paclitaxel (Miyake *et al.*, 2000a),cisplatin (Miyake *et al.*, 2001a) and gemcitabine (Miyake *et al.*, 2004a). This antisense-CLU reagent, OGX-011, has already been entered into Phase I clinical trials together with docetaxel administration in patients with advanced stages of cancer, including prostate, ovarian, renal, lung, bladder, and breast cancers (Chi *et al.*, 2005, 2008). Serum sCLU levels were effectively reduced without serious toxicity. Of the 32 patients who were evaluable, 2 with hormone-refractory prostate cancer had a partial response, 11 had stable disease up to 6 months, and 1 breast cancer patient had a complete response (Chi *et al.*, 2008). Of 14 hormone-refractory prostate cancer patients treated, 3 showed a decline in PSA. Based on these initial successes, a Phase II trial has been instituted. Another independent study of OGX-011 Phase II trial on 15 patients with measurable metastatic breast cancer has been published and again confirmed that the toxicity was no greater than docetaxel alone (Chia *et al.*, 2009). However, although some clinical responses were seen, they were not beyond those expected with docetaxel alone. It is too soon to predict the efficacy of such antisense therapy in cancer patients but the promise of disrupting sCLU to achieve better chemosensitivity to established drugs is still an attractive goal to pursue.

Instead of direct targeting of sCLU, focusing on compounds that can interrupt sCLU gene expression might also be a useful strategy. This area, however, remains unexplored and must await better knowledge of CLU mechanism of induction and expression, and the availability of compounds that can target the identified processes.

In summary, sCLU has emerged as a potent adaptive response to cell stress, either induced by cytotoxic agents, ionizing radiation, or targeted therapy. It is part of the normal cell response to stress as a cytoprotective reaction but in malignant cells, this property works to the advantage of the tumor. Because it confers survival advantage to cancer cells and is readily induced by therapeutic agents, sCLU targeting as a means to chemosensitization toward clinically established drugs could be a potent strategy to overcome drug resistance.

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