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VCP inhibitors induce endoplasmic reticulum stress, cause cell cycle arrest, trigger caspase-mediated cell death and synergistically kill ovarian cancer cells in combination with Salubrinal

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ABSTRACT

Valosin-containing protein (VCP) or p97, a member of AAA-ATPase protein family, has been associated with various cellular functions including endoplasmic reticulum-associated degradation (ERAD), Golgi membrane reassembly, autophagy, DNA repair, and cell division. Recent studies identified VCP and ubiquitin proteasome system (UPS) as synthetic lethal targets in ovarian cancer. Here, we describe the preclinical activity of VCP inhibitors in ovarian cancer. Results from our studies suggest that quinazoline-based VCP inhibitors initiate G1 cell cycle arrest, attenuate cap-dependent translation and induce programmed cell death via the intrinsic and the extrinsic modes of apoptosis. Mechanistic studies point to the unresolved unfolded protein response (UPR) as a mechanism by which VCP inhibitors contribute to cytotoxicity. These results support an emerging concept that UPR and endoplasmic reticulum (ER) stress pathways may be targeted in ovarian cancer as a source of vulnerability. Since prolonged ER stress may result in CHOP-mediated cell death, we tested the hypothesis that VCP inhibitors act synergistically with compounds that enhance CHOP expression. Here, we show that VCP inhibitors act synergistically with Salubrinal, an inhibitor of eIF2α dephosphorylation, by enhancing CHOP expression in ovarian cancer cell lines. Our results provide a proof-of-concept that VCP inhibitors can be used as a single agent and can be synergized with compounds that enhance CHOP expression to induce cell death in ovarian cancer cells.

Abbreviations: AAA-ATPase, ATPase associated with diverse cellular activities; BRCA1/2, breast cancer 1/2; PARP1, poly ADP-ribose polymerase-1; DBeQ, N^2,N^4-dibenzylquinazoline-2,4-diamine; PBS, phosphate buffered saline; EGF, epidermal growth factor; TBS, tris-buffered saline; PERK, protein kinase R-like endoplasmic reticulum kinase; TCGA, the Cancer Genome Atlas; PVDF, polyvinylidene difluoride; IRE1α, inositol-requiring enzyme 1 alpha; CHOP, CCAAT/enhancer-binding protein homologous protein; ATF4, activating transcription factor 4; eIF2α, eukaryotic initiation factor 2 alpha; BiP, binding immunoglobulin protein; Grp78, glucose regulated protein 78; GADD34, growth arrest And DNA-damage-inducible 34.

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1. Introduction

Ovarian cancer affected 239,000 women worldwide in 2012 (Ferlay et al., 2013), and it is the leading cause of death from gynecologic cancer in the United States. Current standard-of-care for advanced-stage ovarian cancer includes debulking surgery followed by adjuvant combination chemotherapy consisting of a platinum agent and a taxane agent (Chien et al., 2013). Although the combination therapy is effective and the initial response rate is over 70%, the majority of patients with advanced disease experience recurrence (Chien et al., 2013). With subsequent re-challenge with platinum-based chemotherapy, most will acquire resistance to chemotherapy and succumb to the disease.

Therefore, it is imperative that novel therapeutic agents be developed to treat ovarian cancer and to extend the effectiveness of platinum-based chemotherapy. Recent advances in cancer therapeutics indicate that genetic defects in cancer can be exploited by synthetic lethality with chemical inhibitors. Synthetic lethality is a concept first reported in Drosophila genetics to describe the lethality to the fly arising from a combination of mutations in two or more genes (Nijman, 2011). This concept was extended as an approach to target cancer-specific mutations to cause lethality in cancer cells and not in normal cells. For example, mutations in BRCA1 and BRCA2 homology recombination (HR) repair genes are common in ovarian and breast carcinomas, and cancer cells harboring these mutations are extremely sensitive to PARP1 inhibition as a result of synthetic lethality (Bryant et al., 2005; Farmer et al., 2005). Accordingly, cancer cells harboring mutations in HR repair genes can be selectively killed by PARP1 inhibitors which cause reduced toxicity to normal cells (Underhill et al., 2011).

This concept was further extended to suggest that mutations in cancer cells create new vulnerabilities that can be exploited for therapeutic benefits (Nijhawan et al., 2012). In support of this concept, several genome-scale genetic screens with short hairpin RNAs (shRNAs) identified components of the endoplasmic reticulum (ER) stress pathway as targets of vulnerability in ovarian cancer (Cheung et al., 2011; Marcotte et al., 2012; Nijhawan et al., 2012). In particular, Valosin-containing protein (VCP), also known as p97 AAA-ATPase, was identified as one of the essential genes in 25 ovarian cancer cell lines compared to 75 non-ovarian cancer cell lines (Cheung et al., 2011). Moreover, VCP was also identified as an essential gene in cyclin E1-overexpressed cisplatin-resistant ovarian cancer cells (Etemadmoghadam et al., 2013). With a barrel-shaped homohexameric configuration, VCP comprises of two AAA-ATPase domains (D1 and D2) and N-terminal domain (Mountassif et al., 2015). Both D1 and D2 domains bind to ATP. However, the D2 domain has been shown to be primarily involved in the VCP ATPase activity. Furthermore, N-terminal domain acts as a primary region for substrate and cofactor binding (Banerjee et al., 2016).

Several studies have associated VCP with various cellular functions including endoplasmic reticulum-associated degradation (ERAD), Golgi membrane reassembly, autophagy and cell division (Deshaies, 2014; Meyer et al., 2002; Seguin et al., 2014). VCP is involved in the extraction of unfolded proteins from the endoplasmic reticulum. Upon extraction, these proteins undergo proteasome-mediated degradation. Cancer cells harbor a plethora of mutations which result in increased load of unfolded proteins required for degradation. Accordingly, VCP and components of the proteasomal degradation pathway are essential for cancer cell survival and in turn present a target of vulnerability in the cancer cell that could be exploited for cancer therapy.

Several groups have recently developed specific inhibitors against VCP (Alverez et al., 2015, 2016; Bursavich et al., 2010; Chou et al., 2010, 2011, 2013, 2014; Fang et al., 2015; Gui et al., 2016; Magnaghi et al., 2013; Polucci et al., 2013; Wijeratne et al., 2016), these efforts were reviewed and summarized elsewhere (Chapman et al., 2015). Among these specific inhibitors, DBeQ was initially developed as a reversible inhibitor of VCP/p97 that compromises protein homeostasis through impairment of both ubiquitin proteasome system (UPS) and autophagic protein clearance. DBeQ targets both D1 and D2-ATPase domains of VCP (Fang et al., 2015). In contrast, ML240, which was derived from the scaffold of DBeQ, targets the D2-ATPase domain (Fang et al., 2015). Another VCP inhibitor NMS-873 is a potent noncovalent, non-ATP-competitive, allosteric inhibitor of VCP and activates unfolded protein response, inhibits autophagy and induces cell death (Magnaghi et al., 2013). Finally, a derivative of ML240 was further developed by Cleave Biosciences to produce orally bioavailable active compound CB-5083 that shows promising preclinical activities (Anderson et al., 2015). Consequently, CB-5083 is currently in two Phase I clinical trials (NCT02243917 and NCT02223598) sponsored by Cleave Biosciences.

Since VCP is identified as an essential gene in ovarian cancer cell lineages and also in Cyclin E1-overexpressed cisplatin-resistant ovarian cancer cell lines, we tested the potential cytotoxic activities of quinazoline-based VCP inhibitors, DBeQ, ML240, and its clinical lead CB-5083 in ovarian cancer cell lines. Moreover, we also determine the potential synergistic drug interactions between VCP inhibitors and Salubrinal, an agent that enhances the expression of CHOP (also known as DDIT3, DNA Damage-Inducible Transcript 3).

2. Materials and methods

2.1. *Reagents*

DBeQ and ML240 were provided by Dr. Frank Schoenen at the University of Kansas (Chou et al., 2013). Salubrinal (CML0951) and tunicamycin (11089-65-9) were purchased Sigma−Aldrich. CB-5083 (S8101) was purchased from Selleckchem. DBeQ, ML240, and CB-5083 were dissolved in dimethyl sulfoxide (DMSO) at the concentration of 50 mM. Salubrinal was dissolved at the concentration of 20 mM in DMSO, and tunicamycin was dissolved at the concentration of 10 mM in DMSO. Dissolved compounds were aliquoted and stored at −80 °C. Stocks were thawed at room temperature and dissolved in appropriate media at selected concentrations immediately before use.

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