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TITLE PAGE

CXL146, a novel 4*H*-chromene derivative, targets GRP78 to selectively eliminate multidrug resistant cancer cells

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RUNNING TITLE PAGE:

CXL146 reduces GRP78 in cancer cells to overcome resistance

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List of non-standard abbreviation

ANOVA	analysis of variance
ER	endoplasmic reticulum
GRP78	78-kDa glucose-regulated protein
MDR	multidrug resistance
MX	mitoxantrone
UPR	unfolded protein response

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Text page	11
Number of tables	1
Number of figures	6
Number of references	35
Words in abstract	247
Words in introduction	535
Words in discussion	678

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ABSTRACT GRP78, an endoplasmic reticulum (ER) chaperone, is a master regulator of the ER stress. A number of studies revealed that high levels of GRP78 protein in cancer cells confer multidrug resistance (MDR) to therapeutic treatment. Therefore, drug candidate that reduces GRP78 may represent a novel approach to eliminate MDR cancer cells. Our earlier studies showed that a set of 4*H*-chromene derivatives induce selective cytotoxicity in MDR cancer cells. In the present study, we elucidated its selective mechanism in four MDR cancer cell lines with one lead candidate (CXL146). Cytotoxicity results confirmed the selective cytotoxicity of CXL146 towards the MDR cancer cell lines. We noted significant overexpression of GRP78 in all four MDR cell lines compared to the parental cell lines. Unexpectedly, CXL146 treatment rapidly and dose-dependently reduced GRP78 protein in MDR cancer cell lines. Using HL60/MX2 cell line as the model, we demonstrated that CXL146 treatment activated the unfolded protein response (UPR) as evidenced by the activation of IRE1-α, PERK and ATF6. CXL146-induced UPR activation led to a series of downstream events, including ERK1/2 and JNK activation, which contributed to CXL146-induced apoptosis. Targeted reduction in GRP78 resulted in reduced sensitivity of HL60/MX2 towards CXL146. Long-term sub-lethal CXL146 exposure also led to reduction in GRP78 in HL60/MX2. These data collectively support GRP78 as the target of CXL146 in MDR treatment. Interestingly, HL60/MX2 upon long-term sub-lethal CXL146 exposure regained sensitivity to mitoxantrone treatment. Therefore, further exploration of CXL146 as a novel therapy in treating MDR cancer cells is warranted.

SIGNIFICANCE STATEMENT Multi-drug resistance is one major challenge to cancer treatment. This study provides evidence that cancer cells overexpress GRP78 as a mechanism to acquire resistance to standard cancer therapies. A chromene-based small molecule, CXL146, selectively eliminates cancer cells with GRP78 overexpression via activating UPR-mediated

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apoptosis. Further characterization indicates that CXL146 and standard therapies complementarily target different populations of cancer cells, supporting the potential of CXL146 to overcome multi-drug resistance in cancer treatment.

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Introduction

Despite the wide range of available cancer therapies and many candidates in the development pipeline, cancer treatment typically suffers from one significant and common challenge – cancer cells can acquire resistance, particularly multi-drug resistance (MDR), through various mechanisms that substantially limits the efficacy of cancer treatment. Therapeutic candidates selectively targeting MDR cancer cells are urgently needed to complement current cancer therapies for better treatment outcome.

Through the combination of rational design and phenotypic screening, we identified and developed a series of *4H*-chromene-based compounds, namely CXL series, which induce selective cytotoxicity towards a range of MDR cancer cells in comparison to the corresponding parental cancer cells and untransformed cells (Aridoss et al., 2012; Bian et al., 2018; Das et al., 2009; Das et al., 2011; Tian et al., 2008). Mechanistically, these compounds induce cell death via apoptosis through an endoplasmic reticulum (ER) calcium signaling pathway instead of the traditional mitochondria pathway (Bian et al., 2018; Bleeker et al., 2013; Hermanson et al., 2009). Most excitingly, MDR cancer cells, upon chronic exposure to CXL compounds, not only failed to acquire drug resistance towards CXL compound but also regained sensitivity towards standard therapies (Das et al., 2013). These unique characteristics strongly support the potential clinical impact of CXL series. The mechanism of action of CXL compounds, however, remains to be fully characterized, particularly an understanding of the molecular basis for the selective cytotoxicity towards MDR cancer cells. Such knowledge is critical to guide its translational evaluation.

GRP78, also referred to as HSPA5 or Bip, is an ER chaperone protein that regulates ER function and is involved in the unfolded protein response (UPR) (Malhotra and Kaufman, 2007). In brief, GRP78 resides in ER lumen to facilitate the proper folding and assembly of newly

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synthesized polypeptides into functional proteins, retain unassembled precursors to the ER, and direct misfolded proteins for degradation. Under normal ER stress, UPR engages adaptive pathways to alleviate ER stress by arresting protein translation, up-regulating chaperones and folding enzymes, and enhancing the degradation of misfolded proteins. Intense or persistent activation of UPR in response to continued ER stress, however, triggers apoptosis, involving three signaling pathways mediated by the key proteins IRE1α, ATF6 and PERK (Kaufman, 1999; Sano and Reed, 2013). IRE1α activation stimulates the stress kinase JNK. ATF6, a transcriptional factor, translocates to the Golgi compartment upon ER stress and is proteolytically cleaved into an active form, which subsequently is translocated to the nucleaus where it activates its target gene transcription. PERK attenuates protein translation by phosphorylating eIF2α. Phosphorylation of eIF2α induces preferential translation of UPR-dependent genes such as ATF4, which then activates the apoptotic pathway through ERK1/2 (Ron and Hubbard, 2008). GRP78 has been reported to be overexpressed in several types of cancer (Gifford et al., 2016; Mozos et al., 2011; Pootrakul et al., 2006; Roller and Maddalo, 2013), some of which are resistant to chemotherapies (Abdel Malek et al., 2015; Gifford et al., 2016; Jagannathan et al., 2015; Mozos et al., 2011; Pootrakul et al., 2006; Pyrko et al., 2007; Reddy et al., 2003; Roller and Maddalo, 2013).

In this study, we provide evidence that four independently developed MDR cancer cell lines, acquired by exposure to different chemotherapeutics, have higher expression of GRP78 compared to their parental cell lines. CXL146 treatment led to the reduction of GRP78 in these cancer cells. CXL146 treatment also activated all three arms of UPR signaling in the MDR cells, contributing to the induction of apoptosis. Targeted depletion of GRP78 via CRISPR knockout support our hypothesis that GRP78 overexpression in MDR cancer cells was responsible for CXL146's selective cytotoxicity. Long-term CXL146 exposure resulted in reduced GRP78 in MDR cancer

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cells and increased the sensitivity of MDR cancer cells to mitoxantrone. These results support a role for GRP78 as a key target for CXL146's selective cytotoxicity towards MDR cancer cells.

Materials and Methods

Cell culture, Chemicals, and Reagents

HL60, HL60/MX2, HL60/DOX, K562, K562/DOX and K562/HHT300 cell lines were purchased from ATCC or provided through our collaborators (Aridoss et al., 2012). HL60/MX2 and HL60/DOX were developed from HL60 upon chronic exposure to sub-lethal mitoxantrone and doxorubicin respectively. K562/DOX and K562/HHT300 were obtained from K562 upon chronic exposure to sub-lethal doxorubicin and homoharringtonine respectively. They were authenticated via the Cell Line Authentication Service provided by Genetica DNA Laboratories (Burlington, NC). They were cultured in RPMI1640 medium supplemented with 10% FBS (Gibco). Antibodies for GRP78, PERK, pIRE1α, IRE1α, ATF6, ATF4, JNK, pJNK, ERK1/2, pERK1/2, cyclin D1, cleaved PARP and anti-rabbit IgG secondary antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-β-actin was purchased from Sigma-Aldrich (St. Louis, MO, USA). CellTiter-Blue reagent was purchased from Promega (Madison, WI, USA). CXL146 was synthesized and characterized following our reported procedures.(Bian et al., 2018)

Cytotoxicity assay

The effect of CXL146 and mitoxantrone on the growth of cancer cell lines were determined using CellTiter-Blue cell viability assay (Promega, G9241). Briefly, HL60, HL60/MX2, HL60/DOX, K562, K562/DOX and K562/HHT300 cells were plated in a 96 well plate ($1x10^4$ cells/well) and treated with CXL146 or mitoxantrone in a series of 1% DMSO dilutions in triplicate. Cells treated

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with 1% DMSO medium served as the control. After 48-h treatment, CellTiter-Blue reagent was added according to the manufacturer protocol with the fluorescence intensity measured typically 1 – 2h after. GI_{50} was calculated by fitting the relative viability of the cells to the compound concentration using a dose-response curve in the GraphPad Prism Software, Inc. (San Diego, CA, USA). A minimum of three biological replicates were performed on different days and data were reported as mean GI_{50} ± SD.

Western blot analysis

Cells with or without treatment were harvested and lysed using RIPA buffer with protease inhibitor cocktail and cleared by centrifugation at 10,000 rpm for 10 min at 4ºC. Protein concentrations were determined by Bio-Rad Protein Assay. Equal amount of protein (50 µg) was electrophoresed on SDS-polyacrylamide gels and transferred onto PVDF membrane. Membranes were blocked with 5% milk in Tris-buffered saline containing Tween-20 before probing with primary antibody according to the instructions of the manufacturer. Subsequently, the membranes were incubated with the corresponding horseradish peroxidase conjugated secondary antibody for 1 h. Protein bands were detected by enhanced ECL reagent (Thermo Scientific), and visualized by BioRad Imaging system. For reprobing, blots were stripped with Restore Western Blot stripping buffer (Thermo Scientific). A minimum of three biological replicates were performed on different days.

CXL146 or mitoxantrone long-term exposure

HL60/MX2 cell lines were exposed to varying concentrations of CXL146 or mitoxantrone or 1.0% DMSO medium for 3 month. The medium was replaced every 3 days with new compound replenished and the cell density was adjusted so that the medium was sufficient for 3-day culturing.

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The cell viability was measured using Cell Titer blue assay. The concentrations of CXL146 or mitoxantrone were adjusted so that there would be no more than 25% growth inhibition during the experimental period (the cells were continuously exposed to sub-toxic levels).

GRP78 down-regulation by CRISPR/Cas9

Three guide RNAs targeting GRP78 (NM_005347.4) were chosen from the Brunello genome-wide CRISPR pooled gRNAs library.(Doench et al., 2016)

GRP78 KO-1 (CAGACGGGTCATTCCACGTG), GRP78 KO-2 (AATGGCAAGGAACCATCCCG) and GRP78 KO-3 (GGTGAGAAGAGACACATCGA) gRNAs were cloned into the lentiviral vector LentiCRISPRv2 (Plasmid #52961, Addgene) using the golden gate assembly strategy as described.(Joung et al., 2017) To generate Lentiviruses for each GRP78 gRNA, LentiCRISPRv2 vector harboring each of the GRP78 gRNA was lipofectamine co-transfected in HEK293T cells with the envelop PMD2.G and packaging psPAX2 plasmid following our standard procedures. HL60-MX2 cells were transduced separately with each of the three GRP78 gRNAs using the spinoculation method. To select the cells that integrated the lentiviral genome (lentiCRISPRv2 harboring the GRP78 gRNA), the transduced cells were cultured in medium supplemented with 2 μg/ml puromycin for 6 days.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Dunnett's test was used for comparisons of the data between different groups. P value ≤ 0.05 was considered statistically significant. All analyses were conducted in GraphPad Prism4 (GraphPad Software, Inc.)

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Results

CXL146 showed preferential cytotoxicity towards MDR cancer cells

The sensitivity of HL60, HL60/MX2, HL60/DOX, K562, K562/DOX and K562/HHT300 cells towards CXL146 or mitoxantrone was evaluated via the CellTiter-Blue assay. As shown in Table 1, MDR cancer cells (HL60/MX2, HL60/DOX, K562/DOX and K562/HHT300) (Aridoss et al., 2012) are less sensitive to mitoxantrone in comparison to the corresponding parental cancer cell lines (HL60 and K562 respectively). In contrast, with CXL146 exposure, all MDR cancer cells were more sensitive to CXL146 relative to the corresponding parental cancer cell lines. These results were consistent with our previous observations that the CXL derivatives selectively kill MDR cancer cells (Aridoss et al., 2012; Bian et al., 2018; Das et al., 2009; Das et al., 2011; Tian et al., 2008).

Characterization of GRP78 proteins in parental and MDR cancer cell lines and the effect of CXL146 treatment

Given the role of GRP78 in modulating ER stress, its overexpression in cancer cells, particularly in the context of MDR, the increased sensitivity of MDR cells to CXL146, and the mechanism of CXL in inducing ER stress (Bleeker et al., 2013; Hermanson et al., 2009), we characterized the levels of GRP78 in the parental and MDR cancer cell lines. GRP78 was overexpressed in all four MDR cancer cell lines relative to the parental control cell lines (Fig. 1A). Since CXL146 showed enhanced cytotoxicity to the MDR cancer cells, we characterized the effect of CXL146 on GRP78 in these cell lines. Upon 24-h exposure, CXL146 treatment dose-dependently and significantly reduced GRP78 levels in all of these cell lines (Fig 1B). Overall, lower concentrations of CXL146 were required to reduce GRP78 in MDR cells as compared to the parental cell lines. It should be

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noted that the CXL146 concentrations that resulted in the loss of GRP78 were comparable to its growth inhibition concentrations in these cell lines (Table 1), corroborating the potential mechanistic relevance.

CXL146 reduced the expression of GRP78 and activated the three arms of UPR in HL60/MX2 cell line

We next used HL60/MX2 as the model to further assess the effect of CXL146. As shown in Fig. 2A, the expression of the GRP78 binding partners, IRE1-α, PERK and ATF6, was substantially higher in HL60/MX2 cells relative to the parental HL60 cells. A 24-h CXL146 treatment dose-dependently increased IRE1-α, p-IRE1-α, PERK and the cleavage of ATF6 (Fig. 2B), consistent with UPR activation. Given that CXL146 rapidly (within min) induces cytosolic calcium increase by blocking ER calcium uptake (Bian et al., 2018; Bleeker et al., 2013), we characterized the time-course effect of CXL146 treatment on GRP78. As expected, CXL146 treatment induced the reduction in GRP78 even with a 4-h exposure and the effect persisted for up to 36 hours (Fig. 2C).

CXL146 induced apoptosis through UPR downstream signaling in HL60/MX2 cells

Among the three UPR arms, PERK mediates eIF2α phosphorylation, leading to the preferential translation of specific mRNAs, one of which encode the ATF4 protein. ATF4 subsequently activates the pro-apoptotic factor and initiates ER stress-associated apoptosis (Blais et al., 2004; Sano and Reed, 2013). Hence we examined the expression of phosphor-eIF2α and ATF4 upon 24-h CXL146 treatment. We observed increased phosphorylation of eIF2α with the reduction of total eIF2α upon CXL146 treatment at various concentrations (Fig. 3A). The level of ATF4 increased upon CXL 146 treatment in a dose-dependent manner as well. CXL146 also dose-dependently

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induced phosphorylation of ERK1/2 (Fig. 3A), which was reported to facilitate ER stress-induced apoptosis. Previous studies revealed that the reduction of GRP78 could lead to the phosphorylation of JNK, a downstream target of IRE1, which also regulates cell death (Lei and Davis, 2003; Urano et al., 2000). In concordance with this, we noticed a dose-dependent phosphorylation of JNK (Fig. 3B). Additionally, GRP78 can interact with procaspase-2 and 7, suppressing their activation (Davidson et al., 2005; Ermakova et al., 2006; Reddy et al., 2003). In agreement with this, as shown in Fig 3A and B, CXL146 treatment reduced the levels of full-length caspase 2 and 7 and increased PARP cleavage and caspase 9 cleaved products. In addition to the induction of the caspase dependent apoptotic pathway, expression of cyclin D1 also plays an important role in the activation of UPR and induces ER stress-mediated apoptosis (Bustany et al., 2015). Furthermore, cyclin D1 is positively regulated by the activation of ERK1/2 (Lavoie et al., 1996). Since we observed the activation of UPR and ERK1/2 by CXL146, we characterized the effect of CXL146 on the expression of cyclin D1. Cyclin D1 expression was induced by CXL146 in a dose dependent manner (Fig. 3B). Lastly, inhibitors specific for ERK1/2 (PD98059) and JNK (SP600125) were able to partially block CXL146-induced PARP cleavage (Fig. 3C), supporting the role of ERK1/2 and JNK activation in CXL146-induced apoptosis.

CXL146 selectively inhibited HL60/MX2 cell proliferation because of GRP78 overexpression

In order to confirm the role of GRP78 in the selective mechanism of CXL146, we targeted the HPSA5 gene encoding GRP78 in HL60/MX2 with three independent sgRNA/Cas9 vectors. We evaluated the pools of HSPA5 knockouts corresponding to each sgRNA instead of individual isolated clones because previous work suggested that the pooled approach provides a robust evaluation of the functional importance of the gene and avoids clone specific effects (Parnas et al.,

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2015; Potting et al., 2018; Shalem et al., 2014). We first characterized GRP78 expression in the pooled knockouts by Western Blotting. Among the three constructs, two (KO-1 and KO-2) were effective while the third construct (KO-3) did not significantly reduce GRP78 in HL60/MX2 (Fig. 4A). We then evaluated their sensitivity to CXL146. As expected, HL60/MX2 cells with reduced levels of GRP78 (KO-1 and KO-2) showed decreased sensitivity to CXL146 while the third construct showed no changes in sensitivity relative to the control cells (Fig. 4B). These results strongly support that CXL146 selectively targets cancer cells with increased levels of GRP78, which are typically drug resistant.

HL60/MX2 reduced GRP78 upon CXL146 chronic exposure and regained sensitivity towards mitoxantrone.

To further explore the relevance of GRP78 as the cellular target of CXL146, HL60/MX2 cells were exposed to a sublethal concentration of CXL146 for three months (mitoxantrone was used as a standard cancer therapy control). We hypothesized that if the high level of GRP78 accounts for the enhanced sensitivity of HL60/MX2 cells to CXL146, CXL146 chronic exposure would select HL60/MX2 cell populations with reduced levels of GRP78 for survival. On the other hand, if GRP78 overexpression confers HL60/MX2 resistance to mitoxantrone, mitoxantrone exposure would select cells with higher levels of GRP78. Indeed chronic exposure of HL60/MX2 cells to CXL146 resulted in cells with reduced GRP78 while chronic exposure of mitoxantrone resulted in further overexpression of GRP78 in HL60/MX2 cells (Fig. 5A). HL60/MX2 cells upon chronic CXL146 treatment regained sensitivity towards mitoxantrone (Fig. 5B), potentially because of the reduction in GRP78.

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Discussion

MDR is one major barrier to the success of cancer treatment. Anticancer agents selectively targeting MDR cancers are critical for more effective cancer management. We developed a series of 4*H*-chromene derivatives, which demonstrated preferential cytotoxicity towards MDR cells (Aridoss et al., 2012; Bian et al., 2018; Das et al., 2009; Das et al., 2011; Tian et al., 2008). Understanding the mechanism of action, particularly selective anticancer activity towards MDR cancer cells, is important to guide its future translational development and potentially clinical evaluation. This study focused on CXL146 as the lead compound to investigate its mechanism in MDR cells.

Among mechanisms that potentially underlie MDR in cancer cells, we focused on GRP78 for two reasons. First, GRP78 has been well documented to be overexpressed in various malignancies. One recent study reported that GRP78 regulates ABC transporter activity in pancreatic cancers to confer drug resistance (Dauer et al., 2018). As ABC transporter up-regulation is one major MDR mechanism, GRP78 overexpression may be a general mechanism of cancers cells to acquire MDR. Our CXL compounds demonstrated selective cytotoxicity towards a wide range of MDR cancer cells (Aridoss et al., 2012; Bian et al., 2018), suggesting that they may be targeting a common MDR mechanism. Second, our earlier studies suggest that ER stress and cytosolic calcium influx contribute to the mechanism of action for CXL compounds (Bian et al., 2018; Bleeker et al., 2013; Hermanson et al., 2009). GRP78 is a key protein on ER in regulating ER stress response and buffering ER calcium (Hammadi et al., 2013; Leustek et al., 1991). These together suggest that GRP78 is a logical target candidate of CXL compounds.

In this study, we first showed that GRP78 is highly expressed in four MDR cell lines compared to their parental cell lines. These MDR cell lines were selected for study because they have

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increased sensitivity towards CXL146 although they are resistant to standard therapies, such as mitoxantrone. Interestingly, CXL treatment resulted in a rapid and dose-dependent reduction in GRP78, providing the first piece of evidence that GRP78 is mechanistically relevant to CXL146. Although CXL146 treatment led to a dose-dependent reduction of GRP78 in both parental and MDR cancer cells, the concentrations of CXL146 needed to induce the reduction in GRP78 in MDR cells were generally lower than those for the parental cell lines. These findings are consistent with the preferential cytotoxicity of CXL towards MDR cancer cell lines and support a role for GRP78 in the preferential cytotoxicity of CXL compounds to MDR cells. Decreased expression of GRP78 via CRISPR Cas9 targeting indeed reduced the sensitivity of HL60/MX2 to CXL146 treatment, providing evidence that GRP78 is essential for the selective mechanism of CXL146. Furthermore, natural selection data demonstrated that GRP78 is integral to the cytotoxicity of CXL146 as chronic sub-lethal exposure of HL60/MX2 to CXL146 selected cells with reduced GRP78 level. These acute, chronic and genetic findings collectively support that GRP78 is necessary for the MDR phenotype in the studied cells and that CXL146 selectively kills cancer cells with elevated GRP78levels, demonstrating its unique anti-cancer potential. Of note, the HL60/MX2 cells selected by sub-lethal CXL146 exposure showed increased sensitivity towards mitoxantrone, consistent with our earlier results (Das et al., 2013), providing strong evidence that CXL146 may be complementary to standard therapies to mitigate the risk of MDR development in cancer treatment.

Using HL60/MX2 as the MDR model, we showed that in addition to GRP78 expression, its partners (IRE1α, ATF6 and PERK) were also highly expressed. Importantly, CXL146 treatment impaired the function of GRP78 to sequester IRE1α, ATF6 and PERK, and activated all of the three UPR transducers pathways. Several down-stream markers provided further evidence that

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CXL146 treatment activated UPR (Lei and Davis, 2003; Urano et al., 2000). Based on the fact that small molecules could bind to the ATPase domain of GRP78 and facilitate GRP78dissociation from its UPR partners, CXL146 may target GRP78 via the same mechanism. Further studies are required to verify this hypothesis.

In summary, our results showed that CXL146 effectively reduces GRP78 expression, which is commonly up-regulated among various malignancies and necessary for MDR in some cancer cells. Importantly, CXL146 and standard therapies appear to target different populations of cancer cells on the basis of the GRP78 levels. CXL146 therefore may be complementary to standard cancer therapies for effective cancer management. CXL146 has also demonstrated acceptable pharmacokinetics *in vivo*. Its preferential anticancer potential towards MDR cancer cells will be evaluated *in vivo* to validate its anticancer potential.

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Authorship Contribution

o *Participated in research design*: Tengfei Bian, Abderrahmane Tagmounti, Christopher Vulpe, Kavitha Chandagirikoppal Vijendra, Chengguo Xing

o *Conducted experiments*: Tengfei Bian, Abderrahmane Tagmounti, Kavitha Chandagirikoppal Vijendra

o *Contributed new reagents or analytic tools*: N/A

o *Performed data analysis*: Tengfei Bian, Abderrahmane Tagmounti, Christopher Vulpe, Kavitha Chandagirikoppal Vijendra, Chengguo Xing

o *Wrote or contributed to the writing of the manuscript*: Tengfei Bian, Abderrahmane Tagmounti, Christopher Vulpe, Kavitha Chandagirikoppal Vijendra, Chengguo Xing

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FOOTNOTES

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Legends for Figures

Fig. 1. A. Expression of GRP78 in different MDR and parental cancer cell lines; B. CXL146 treatment resulted in a dose-dependent reduction of GRP78 in all cell lines tested with lower concentrations of CXL146 in MDR cell lines relative to the corresponding parental cell lines. These images are representative of at least three independent replicates.

Fig. 2. A. Characterization of GRP78 and other UPR markers in HL60 and HL60/MX2 cell lines; B. CXL146 treatment activated the three arms of UPR in HL60/MX2 cells; C. CXL146 rapidly induced GRP78 reduction in HL60/MX2 cells. These images are representative of at least three independent replicates.

Fig. 3. CXL146 activated UPRs and induced apoptosis that could be partially blocked by ERK1/2 and JNK inhibition. These images are representative of at least three independent replicates.

Fig. 4. A. Confirmation of GRP78 down-regulation via CRISPR/Cas9 in HL60/MX2 cell line. B. Relative sensitivity of HL60/MX2 cell lines of varied GRP78 levels towards CXL146. The results are representative of at least three independent replicates.

Fig. 5. A. The effect of long-term exposure of CXL146 and mitoxantrone on GRP78 protein level in HL60/MX2. B. The relative sensitivity of these cell lines towards mitoxantrone. The results are representative of at least three independent replicates.

Fig. 6. Proposed mechanism of action for CXL146.

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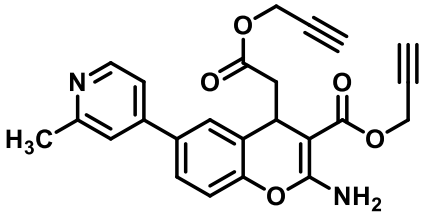
Table 1. GI_{50}s of CXL146 and mitoxantrone towards parental and MDR cancer cell lines



CXL146

mitoxantrone

Cell line	GI_{50} (μM)	Selectivity	GI_{50} (μM)	Selectivity
HL60	0.48 ± 0.07	1	0.0040 ± 0.0003	1
HL60/MX2	0.17 ± 0.03	**2.8**	0.18 ± 0.01	**0.02**
HL60/DOX	0.30 ± 0.07	**1.6**	0.007 ± 0.001	**0.57**
K562	0.75 ± 0.09	1	0.31 ± 0.05	1
K562/DOX	0.49 ± 0.11	**1.5**	0.89 ± 0.04	**0.35**
K562/HHT300	0.51 ± 0.03	**1.5**	3.8 ± 0.2	**0.08**

Selectivity is the ratio of GI_{50} of the parental cell line to that of the MDR cell line

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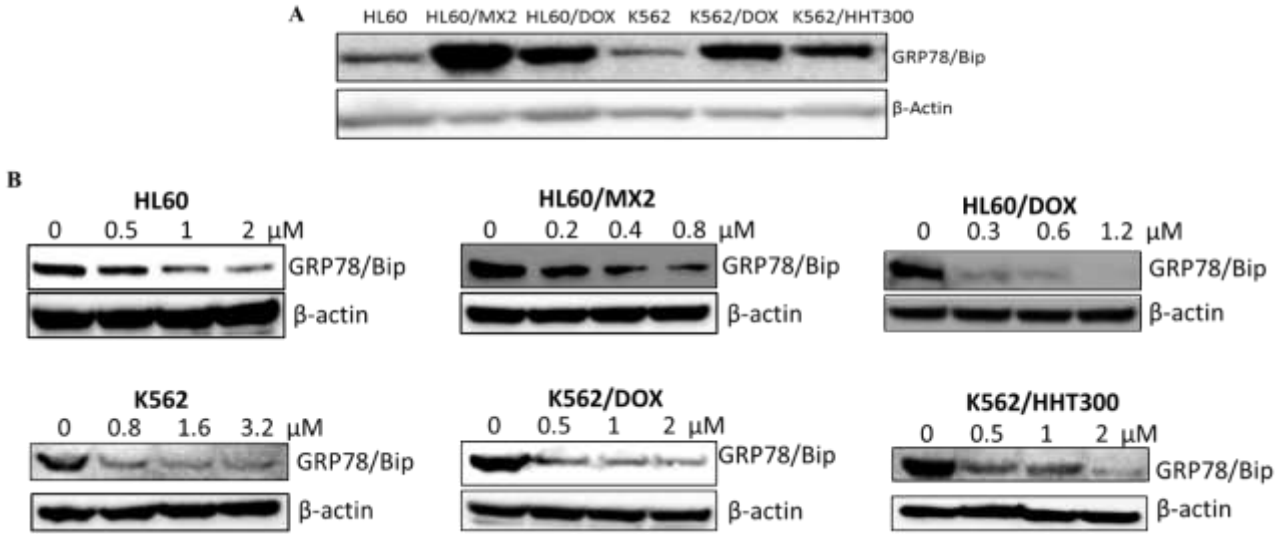

Fig. 1.

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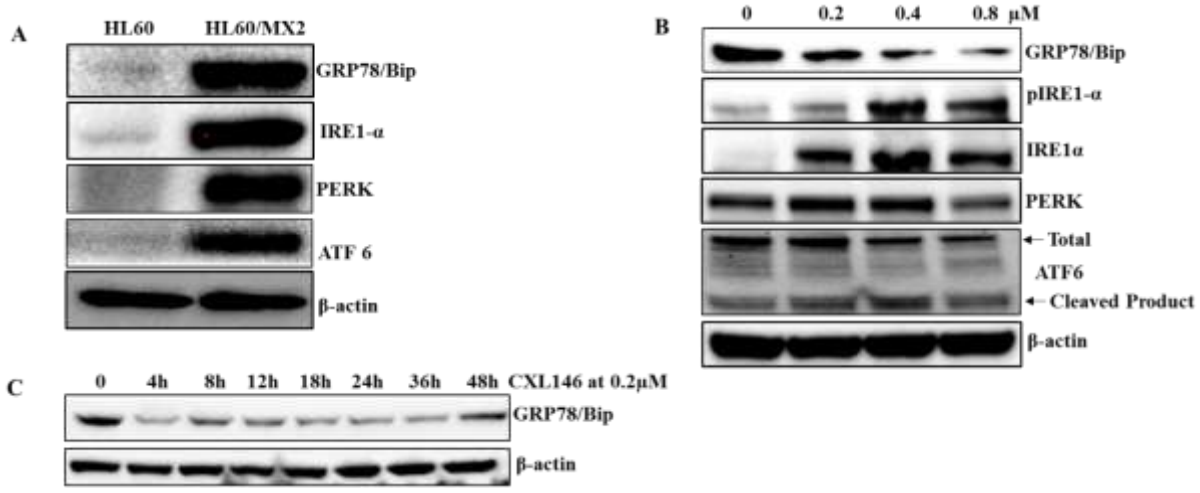

Fig. 2.

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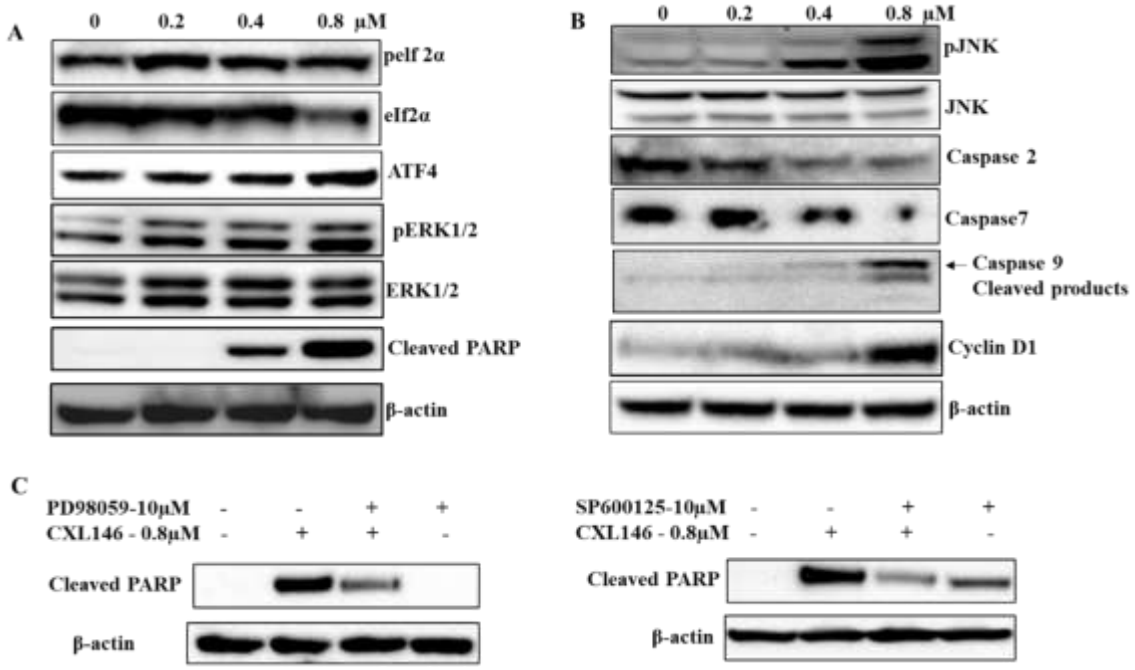

Fig. 3.

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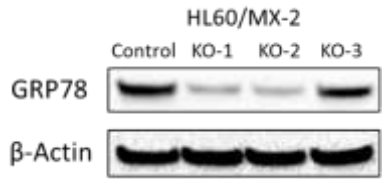
A.



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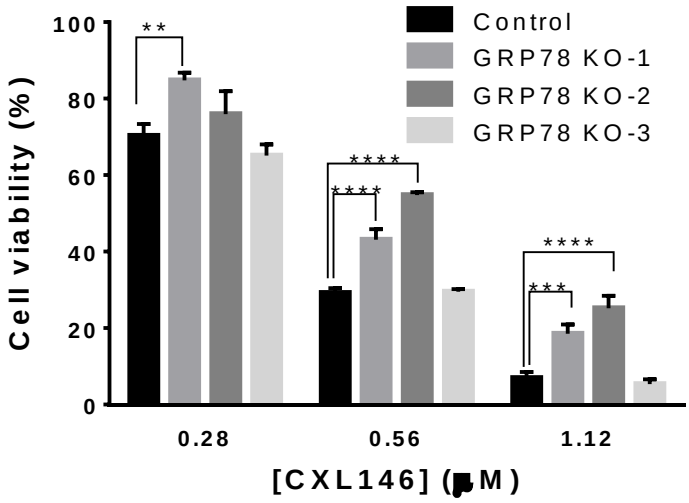

Fig. 4.

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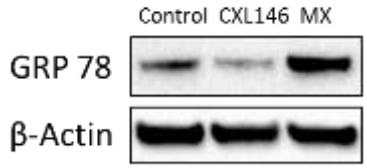

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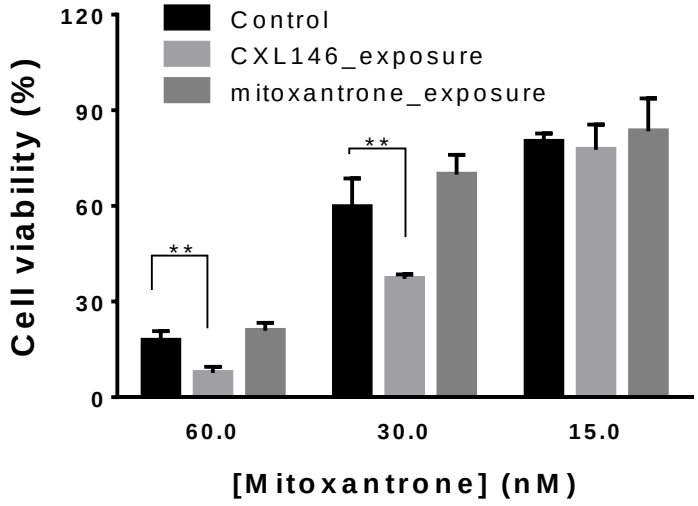

Fig. 5.

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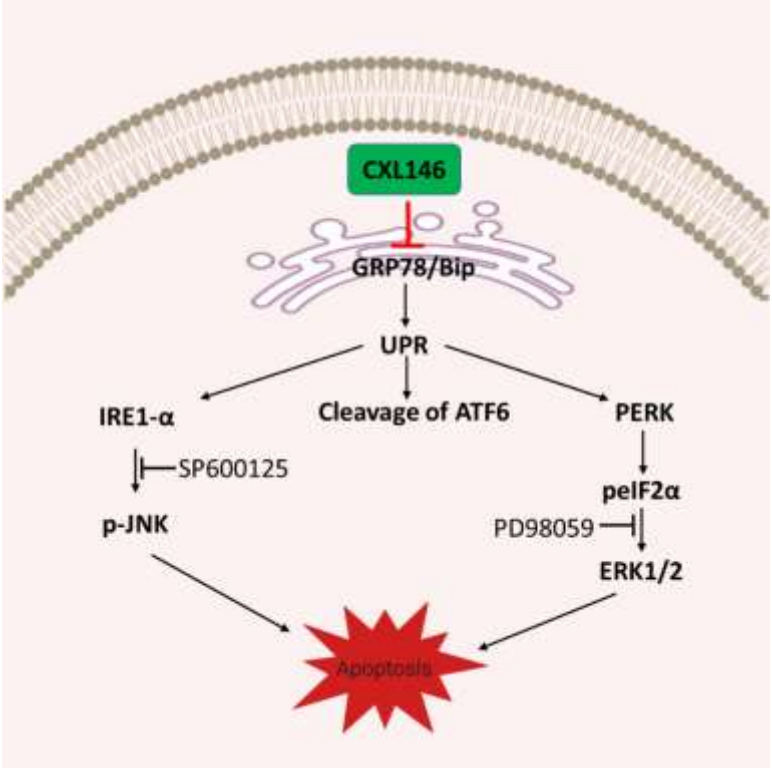

Fig. 6.

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