Inhibition of GRP78 sensitizes colorectal cancer cells to paclitaxel-induced apoptosis by activation of caspase-4

Nizar Mhaidat, Saied Jaradat, Ahmad Aldaher, and Abdulhameed Ghabkari

Abstract**—** Resistance of colorectal cancer cells to paclitaxel-induced apoptosis is largely mediated by the activation of MEK/ERK signalling pathway. Inhibition of MEK/ERK pathway sensitized CRC cells to paclitaxel-induced apoptosis by down-regulation of GRP78. In the present study, we report that induction of apoptosis by paclitaxel when GRP78 is down-regulated involves activation of the caspase cascade. In cells, where GRP78 is inhibited, paclitaxel induced activation of caspase-3, caspase-4, and caspase-9. Caspase-4 seemed to be the apical caspase in that caspase-4 activation occurred before activation of caspase-9 and caspase-3. Moreover, activation of caspase-4 was upstream of the mitochondria and its inhibition led to the inhibition of mitochondrial membrane permeability (MMP) and caspase-9 activation. Furthermore, co-immunoprecipitation studies revealed that GRP78 is physically associated with caspase-4 before and after treatment with paclitaxel. These results indicate that GRP78 might be a novel mechanism underlying resistance of CRC cells to microtubule-targeting drugs by binding to and inhibition of caspase-4. Combination of compounds capable of suppressing GRP78 might be a golden approach for improving the effectiveness of taxanes in treatment of CRC.

Keywords **—** Apoptosis, Colorectal Cancer, GRP78, Paclitaxel, UPR.

I. INTRODUCTION

A A number of cellular stress conditions, such as hypoxia, alterations in glycosylation status, and disturbances of calcium flux, lead to accumulation of unfolded and/or misfolded proteins in the endoplasmic reticulum (ER) lumen and cause so-called ER stress [1-3]. The ER responds to the stress conditions by activation of a range of stress-response signaling pathways that couple the ER protein folding load with the ER protein folding capacity and is termed the unfolded protein response (UPR) [1-3].

Manuscript received March 8, 2011: This work was supported by Jordan University of Science & Technology Grant 225-2009.

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The UPR of mammalian cells is initiated by three ER transmembrane proteins-activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1) and double stranded RNA-activated protein kinase-like ER kinase (PERK) [1–3]. Under unstressed conditions, the luminal domains of these sensors are occupied by the ER chaperon glucose-regulated protein 78 (GRP78) [1–3]. Upon ER stress, sequestration of GRP78 by unfolded proteins activates these sensors by inducing phosphorylation and homodimerization of IRE1 and PERK, and relocation and proteolytic cleavage of ATF6 [1–3].

Caspase-12 is thought to be a key mediator as caspase-12–deficient mouse cells are partially resistant to ER stress-induced apoptosis [4]. However, caspase-12 is expressed only in rodents. Its human homologue is silenced by several mutations during evolution [5]. Human caspase-4 has been shown to fulfill the function of caspase-12and plays an important role in ER stress-induced apoptosis of human neuroblastoma and HeLa cells [6]. Moreover, the c-Jun NH2-terminal kinase (JNK), the transcription factor CAAT/enhancer binding protein homologous protein (CHOP), and deregulation of Bcl-2an d inhibitor of apoptosis protein family members have all been suggested to play roles in ER stress-induced apoptosis [7, 8, 9-12].

There is increasing evidence to show that the UPR is activated in various solid tumors, perhaps due to nutrient deprivation and hypoxia. Elevated expression of GRP78 has been reported in several cancers, such as breast cancer and prostate cancer [13-16]. Moreover, GRP78 expression has been shown in some cases to be associated with tumor development and growth and correlated with resistance to certain forms of chemotherapy. It seems that some cancer cells may have adapted to ER stress by activation of the UPR without resulting in apoptosis [14, 15, 17]. The central feature of this adaptive response has been suggested to be maintenance of expression of proteins that facilitate survival, such as GRP78 [18]. In addition, other survival signaling pathways, such as the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK pathways, may also play roles in counteracting the apoptosis-inducing potential of ER stress [13, 19].

In the present study we examined the potential of the ER chaperon GRP78 in regulation of the sensitivity of CRC cells to paclitaxel-induced apoptosis. We show that GRP78 is physically associated with caspase-4 before and after treatment with paclitaxel. Down-regulation of the GRP78 using siRNA pool of GRP78 sensitizes CRC cells to paclitaxel-induced apoptosis, at least in part, by activation of caspase-4.

II. Materials and Methods

A. Cell Lines

Human CRC cell lines Colo205, SW480, SW620, HT29, HCT116, and WiDr were generously provided by Dr. Rick F. Thorne (University of Newcastle, Australia) and were cultured in DMEM containing 10% FCS (Bio Whittaker, Verviers, Belgium).

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B. Chemicals and Reagents

Paclitaxel was purchased from Sigma-Aldrich (USA) and stored as a 20mM solution in Dimethyl sulfoxide (DMSO) with a final concentration of 0.1% (v/v), which did not contribute to toxicity, at –80 °C and diluted with the DMEM medium prior the use. The propidium iodide (PI) and the mouse monoclonal antibody against caspase-4 were purchased from Sigma-Aldrich (Sigma-Aldrich, USA). The rabbit MAbs against GRP78/Bip was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The cell-permeable pan caspase inhibitor z-VAD-fmk, the caspase-3 specific inhibitor z-DEVD-fmk, the caspase-9 specific inhibitor z-LEHD-fmk, the caspase-8 specific inhibitor z-IETD-fmk, and the caspase-4 specific inhibitor z-LEVD-fmk were purchased from R&D System (Minneapolis, MN). The caspase substrates were purchased from Calbiochem (La Jolla, CA).

C. Apoptosis

Quantitation of apoptotic cells by measurement of sub-G1 DNA content using the PI method was carried out as described elsewhere [20].

D. Protein Expression Analysis

Cells were cultured to 80% confluence. The cells were trypsinised and washed with medium twice followed with cold PBS once. Cells were then lysed with a Triton X-100 based lysis buffer (10% Triton X-100, 10% glycerol, 150mM NaCl, 20mM Tris [pH=7.5], 2mM ethylene diamine tetraacetic acid [EDTA], 1mM phenylmethylsulphonyl fluoride [PMSF], 10μg/ml aprotinin, and 10μg/ml leupeptin). Cell lysates were then transferred into microcentrifuge tubes and after 1 hour on ice were centrifuged at 13,000 rpm for 30 minutes at 40C. The protein content of cell extracts was determined by the Bradford assay (Bio-Rad). A total of 20-30 μg of protein was electrophoresed on 10-15% SDS-PAGE gels and transferred to nitrocellulose membranes. Membranes were blocked, incubated with primary Abs at the appropriate concentration, and subsequently incubated with horseradish peroxidase-conjugated goat anti- rabbit IgG or goat anti-mouse IgG (1:3000 dilutions; Bio-Rad). Labeled bands were detected by Immun-StarTM HRP Chemiluminescent Kit, and images were captured. The intensity of the Bands was quantitated with the Bio-Rad VersaDocTM image system. The relative expression of certain protein was determined by dividing the densitometric value of the test protein by that of the control (GAPDH).

E. Small RNA interference (siRNA)

The siRNA constructs used were obtained as the siGENOME SMARTpool reagents (Dharmacon, Lafayette, CO) and the siGENOME SMARTpool GRP78 (M-008198-01). The non-targeting siRNA control, SiConTRolNon-targeting siRNA pool (D-001206-13-20) was also obtained from Dharmacon. Transfection of siRNA pools was carried out as described previously [20].

F. Mitochondrial Membrane Potential ($\Delta\Psi m$)

Tumor cells were seeded at $1x10^5$ cells/well in 24-well plates and allowed to reach exponential growth for 24 hours before treatment. JC-1 staining was performed according to the manufacture's instructions (Molecular Probes, Eugene, OR). Briefly, adherent cells and non-adherent cells were collected and washed with PBS. Cells were then incubated with 10μg/ml of JC-1 in warm PBS at 37oC for 15 minutes. After washing with PBS, the cells were analyzed using a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA). Cells with polarized mitochondria presented in the upper-right quadrant of the dot plot due to the formation of JC-1 aggregates, which emit orange fluorescence (590nM) when excited at 488nM. Cells with depolarized mitochondria emit green fluorescence (530nM) and are visualized in the lower-right quadrant in the dot plot [21].

H. Detection of XBP1 mRNA splicing.

The method used for detection of unspliced and spliced XBP1 mRNAs was as described previously [21]. Briefly, reverse transcription-PCR (RT-PCR) products of XBP1 mRNA were obtained from total RNA extracted using primers 5'-cggtgcgcggtgcgtagtctgga- 3'(sense) and 5'-tgaggggctgagaggtgcttcct-3' (antisense). Because a 26-bp fragment containing an ApaLI site is spliced on activation of XBP1 mRNA, the RT-PCR products were digested with ApaLI to distinguish the active spliced form from the inactive unspliced form. Subsequent electrophoresis revealed the inactive form as two cleaved fragments and the active form as a noncleaved fragment.

J. Caspase activity assay.

Measurement of caspase activities by fluorometric assays was done as described previously [21]. The specific substrates z-DEVD-AFC, Ac-LEVD-AFC, and z-LEHD-AFC were used to measure caspase-3, caspase-4, and caspase-9 activities, respectively (Calbiochem).The generation of free AFC was determined using FLUOstar OPTIMA (Labtech) set at an excitation wavelength of 400 nm and an emission wavelength of 505 nm.

K. Immunoprecipitation

100 µL of lysates were precleared by incubation with 20 µL of a mixture of protein A-Sepharose and protein G-Sepharose packed beads (Santa Cruz Biotechnology) in a rotator at 40C for 2h and then with 20 µL of fresh packed beads in a rotator at 4^0C overnight. Twenty micrograms of anti-GRP78 antibody or control immunoglobulin were then added to the lysate and rotated at 4^0C for 2h. The beads were then pelleted by centrifugation and washed five times with ice-cold lysate buffer before elution of the proteins from the beads in lysate buffer at room temperature for 1 h. The resulted immunoprecipitates were then subjected to SDS-PAGE and Western blot analysis.

G. Statistical Analysis

Data are expressed as mean ± SE. The statistical significance of intergroup differences in normally distributed continuous variables was determined using Student's t-test. *P* values ≤ 0.05 were considered statistically significant. P values ≤ 0.05 and ≤ 0.001 are indicated by * and **, respectively.

III. RESULTS

Previous studies have shown that paclitaxel induces the endoplasmic reticulum (ER) stress response in CRC cells [20]. Inhibition of MEK/ERK pathway sensitized CRC cells to paclitaxel-induced apoptosis by down-regulation of GRP78 [20]. To study if CRC cells may express increased levels of GRP78, we examined GRP78 expression in a panel of CRC cell lines using immunoblotting analyses. Results showed that CRC cell lines expressed varying levels of GRP78 (Fig.1A). Furthermore, we evaluated the expression of another commonly used indicator of UPR activation, the spliced XBP1, by the PCR. Spliced XBP1was observed in all CRC cells but to varying extents being the highest levels in HCT116 cells followed by SW620, HT29, and SW480 CRC cells and the least in Colo205 and WiDr cells (Fig.1B).

We studied the importance of GRP78 in protecting CRC cells from paclitaxel-induced apoptosis by down-regulating GRP78 expression in CRC cells using small interfering RNA molecules. Treatment of SW480 cells with paclitaxel induced a time-dependent increase in the protein levels of GRP78 (Fig.2A).

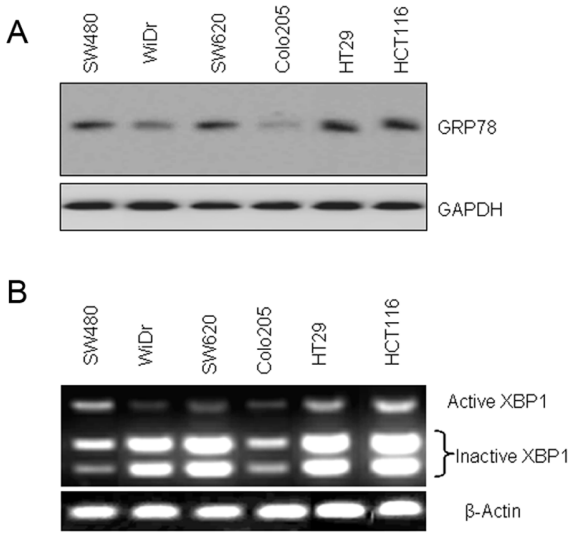

Fig.1: UPR status in CRC cells. (*A*) GRP78 in a panel of CRC cell lines. Whole cell lysates were subjected to Western blot analysis. (*B*) RT-PCR products of XBP1 mRNA from CRC cells digested with ApaLI for 90 min followed by electrophoresis.

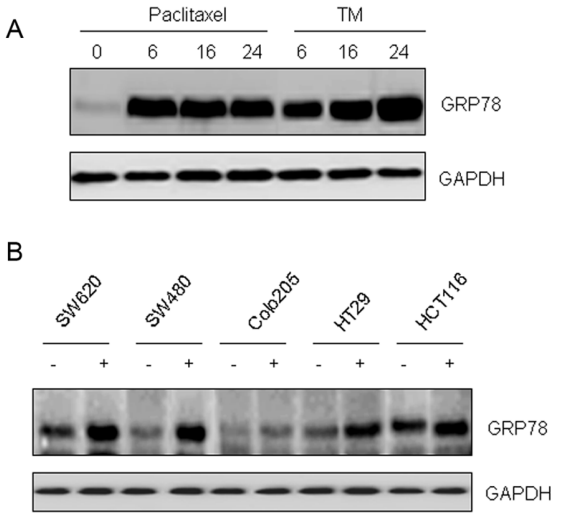

Fig.2: Paclitaxel induces UPR in CRC cells. (*A*) SW480 cells were treated with paclitaxel (40µM) or TM (1µM) for the indicated time periods. Whole cell lysates were subjected to Western blot analysis. (*B*) SW480 cells were treated with paclitaxel (40µM) for 16 hours. Whole cell lysates were subjected to Western blot analysis. Western blot analysis of GAPDH levels was included to show that equivalent amounts of protein were loaded in each lane. Data are representative of two individual experiments.

Tunicamycin (TM) at 3µM was used as a positive control. Similarly, treatment of a panel of CRC cell lines with paclitaxel resulted in varying degrees of increase in GRP78 protein levels among different CRC cells (Fig.2B). Down-regulation of GRP78 levels significantly ($p \leq 0.05$) sensitized SW480 cells to paclitaxel-induced apoptosis (Fig.3A). We next examined whether the variable expression of GRP78 levels is correlated with sensitivity to paclitaxel. Fig.3B

indicates that the expression of GRP78 was inversely correlated with the degree of paclitaxel-induced apoptosis (R=0.9121).

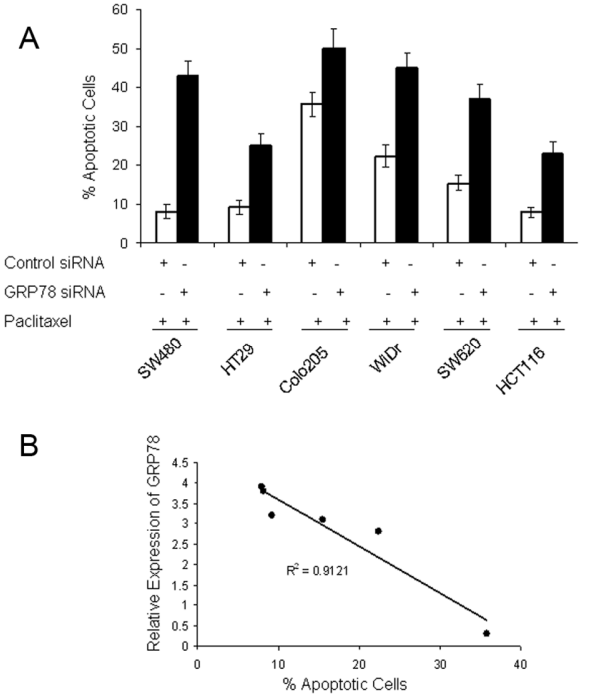

Fig.3: GRP78 antagonizes paclitaxel-induced cytotoxicity in CRC cells. (*A*) Down-regulation of GRP78 expression increases paclitaxel-induced apoptosis of SW480. Cells were transfected with either a non-targeting siRNA (Control siRNA) or with a GRP78 specific siRNA sequence (Darmahcon) at 100nM for 24 hours. Transfected cells were then treated with paclitaxel for another 48 hours before harvest. Cells were analysed for apoptosis using the propidium iodide method. The data are representative of three individual experiments. (*B*) Correlation between the relative expression of GRP78 and the levels of paclitaxel-induced apoptosis in a panel of CRC cell lines. GRP78 relative expression was determined by dividing the densitometric value of the test protein by that of the GAPDH control. Regression analyses were carried out in a Macintosh computer using the StatView software.

We next examined if sensitization of CRC cells to paclitaxel-induced apoptosis by inhibition of GRP78 depends on the caspase cascade. After the down-regulation of GRP78 using siRNA, SW480 cells were treated with the general caspase inhibitor z-VAD fmk 1 hour before the addition of paclitaxel for another 48 hours. Fig.4A shows that z-VAD-fmk markedly inhibited apoptosis induced by paclitaxel in cells where GRP78 was down-regulated, suggesting that the caspase cascade plays a determining role in this sensitization.

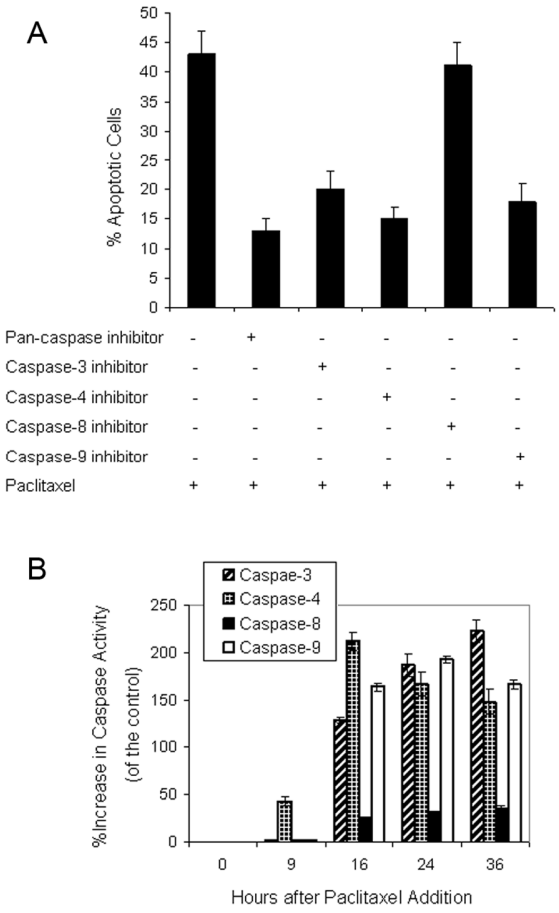

Fig.4: Paclitaxel-induced apoptosis is caspase-4-dependent. (*A*) SW480 cells were pre-treated with the pancaspase inhibitor, z-VAD-fmk (20μM), the caspase-3 inhibitor, z-DEVD-fmk (30μM), the caspase-4, z-LEVD-fmk (30μM), the caspase-8 inhibitor, z-IETDfmk (30μM), or with the caspase-9 inhibitor, z-LEHD-fmk (30μM), 1 hour before paclitaxel (40μM) for 48 hours. Apoptosis was measured by the propidium iodide method using flow cytometry. The data shown are the mean ± SE of three individual experiments. (*B*) SW480 cells were treated with paclitaxel for the indicated time points before cell lysate was analysed for caspase activation using caspase-specific fluorescent AFC substrates. The percentage of increase of caspase activity was calculated as the increase in AFC fluorescence with paclitaxel treatment minus background fluorescence divided by AFC fluorescence without paclitaxel treatment minus the background. Bars indicate mean of three individual experiments ± SE.

To further elucidate the caspases involved, we studied the effects of specific inhibitors against caspase-3, z-DEVD-fmk, caspase-4, z-LEVD-fmk, caspase-8, z-IETD-fmk, and caspase-9, z-LEHD-fmk, on GRP78 down-regulation-mediated sensitization of SW480 cells to paclitaxel-induced apoptosis. As shown in Fig.4A, the inhibitor against caspase-4, caspase-9, or caspase-3 inhibited apoptosis induced by paclitaxel in the GRP78-knocked down SW480 cells to varying degrees. In contrast, the inhibitor against caspase-8 exhibited only minimal inhibitory effects (Fig.4A). These observations suggest that caspase-4, caspase-9, and caspase-3 are involved

in sensitization of CRC cells to paclitaxel-induced apoptosis by GRP78 down-regulation.

Activation of caspase-4, caspase-9, and caspase-3 in SW480 cells after down-regulating GRP78 using siRNA was also confirmed in fluorometric assays detecting activities of the caspases by specific substrates in whole cell lysates as shown in Fig.3B. Caspase-4 appeared to be activated first and to a higher degree compared to other caspases at 9 hours following treatment of SW480 cells with paclitaxel. Activated caspase-3 correlated well with activation of caspase-9. Activation of caspase-8 was delayed and became active after caspase-3 activation indicating that caspase-8 was unlikely to be an initiator of paclitaxel-induced apoptosis.

It is conceivable that all apoptotic pathways including the extrinsic, mitochondrial or intrinsic, and the endoplasmic reticulum pathway are mediating apoptotic events by releasing apoptogenic molecules form the mitochondria such as cytochrome c and apoptosis-inducing factor (AIF) [21]. In order to identify the involvement of mitochondria in the apoptotic pathway being activated in response to paclitaxel in cells where GRP78 is down-regulated, changes in the mitochondrial membrane potential (MMP) were studied using a fluorescent cationic dye 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzamidazolocarbocyanin iodide, known as JC-1 [22].

In healthy cells, JC-1 exists as a monomer in the cytosol (FL1 positive; green) and also accumulates as aggregates in the mitochondria (FL2 positive; red). In apoptotic cells, JC-1 exists exclusively in monomer form and produces a green cytosolic signal [22]. As shown in Fig.5A, paclitaxel induced mitochondrial depolarization detected at 12 hours in SW480 cells and peaked at 48 hours. To explore the role of caspase-4 in paclitaxel-induced apoptosis, caspase-4 specific inhibitor was used. As shown in Fig.5B, inhibition of caspase-4 significantly impaired MMP changes induced by the drug indicating that activation of caspase-4 occurred upstream of the mitochondia.

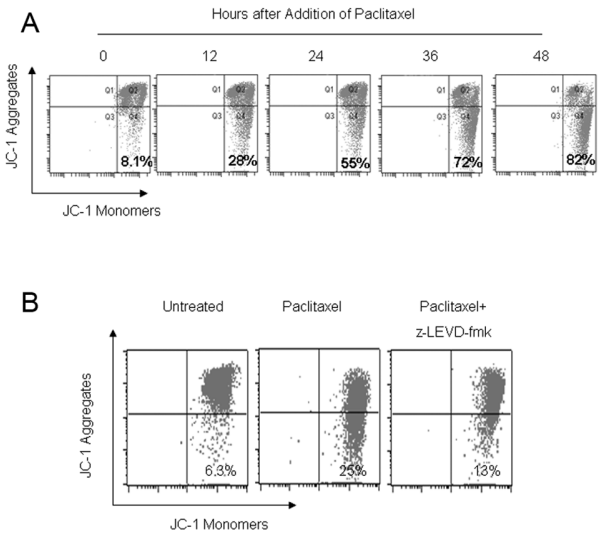

Fig.5: Paclitaxel induces caspase-4 dependent mitochondrial membrane potential (MMP) changes of CRC cells. (*A*) SW480 cells were treated with paclitaxel at 40μM for the indicated time periods followed by measurement of ΔΨm using JC-1 in flow cytometry. The numbers in the lower, right panel represent the percentage of cells with reduced mitochondrial potential as indicated by disappearance of JC-1 aggregates and appearance of JC-1 monomers. The data shown are representative of three individual experiments. (*B*) Caspase-4 is upstream of paclitaxel-induced changes in MMP. SW480 cells were treated with or without caspase-4 inhibitor for 1 hour before the addition of paclitaxel at 40μM for another 48 hours followed by measurement of ΔΨm by JC-1 using flow cytometry. The data shown are representative of three individual experiments.

Moreover, we studied if there is a physical association between caspase-4 and GRP78. As shown in Fig.6, the endogenous caspase-4 could be coimmunoprecipitated with endogenous GRP78.

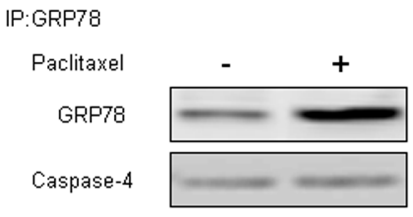

Fig.6: GRP78 is physically associated with caspase-4. Whole cell lysates from SW480 cells with or without treatment with paclitaxel were subjected to immunoprecipitation with GRP78 antibody. The resulting precipitates were analyzed by Western blotting for expression of GRP78 and caspase-4 Data are representative of two experiments.

IV. Discussion

CRC is a disease in which the tumor growth is found in

tissues of the colon and/or rectum. It is the third most common malignancy in the developed Western countries and represents the third leading cause of death from cancer in the United States after lung cancer. Resistance of human CRC cells to the available chemotherapeutic agents is considered a major obstacle to the successful treatment. The purpose therefore of a new approach in treatment of CRC comes from the identification of the mechanisms employed in induction of apoptosis by chemotherapy and the possible resistance mechanisms in CRC cells against chemotherapy-induced apoptosis.

Constitutive activation of Ras/Raf/MEK/ERK signaling is a hallmark of many human cancers such as breast, lung, colorectal cancers, and melanoma [11, 20, 23, 24]. ERK1/2 lies downstream of a group of kinases including protein kinase C (PKC), Raf-1, and MEK1. On stimulation by extracellular signals, they are successively activated by phosphorylation [24]. Previous studies have shown that MEK/ERK signaling pathway is associated with suppression of apoptosis [23, 26, 27]. Furthermore, activation of the MAPK superfamily has been found during ER stress [11, 28, 29]. Activation of MEK/ERK pathway has been found to govern the cell survival during ER stress-induced apoptosis [11, 28]. Recently, it has been shown that the ER chaperone GRP78 expression contributes to antiapoptotic effects and chemotherapy resistance in many cancers [13, 16, 20]. The levels of GRP78 were correlated well with ERK1/2 pathway activation in renal epithelial cells [30].

Investigation of the mechanism involved in the MEK/ERK-mediated inhibition of ER stress-induced apoptosis led to focus on GRP78, which is known to inhibit apoptosis by multiple mechanisms [13]. Our previous findings showed that the enhancement of paclitaxel-induced apoptosis by inhibition of MEK was closely associated with blockage of GRP78 up-regulation, strongly suggest that GRP78 plays a role in MEK/ERK-mediated inhibition of apoptotic signaling in cells subjected to ER stress [20]. Inhibition of MEK/ERK signaling with U0126 or by siRNA knockdown of MEK1 resulted in a decrease in the levels of expression of GRP78 indicating that MEK/ERK pathway might be upstream regulator of GRP78 [20].

The results above seem to provide several new insights into the apoptosis resistance mechanisms that inhibition of MEK/ERK may target in CRC. They show that cultured CRC cell lines do not undergo significant apoptosis when exposed to ER stress induced by paclitaxel. However, ER stress-induced apoptosis is readily triggered when the MEK/ERK pathway is inhibited by either a MEK inhibitor or MEK1 siRNA. This result is of particular interest in that it raises the possibility that constitutive activation of the MEK/ERK pathway in CRC cancer may lay a basis for adaptation of the cells to ER stress conditions. This is mediated, at least in part, by activation of caspase-4 that leads to subsequent apoptosis.

The inhibitory effect of caspase-4 inhibitor on sensitization of CRC cancer cells to paclitaxel-induced apoptosis when MEK was inhibited seemed to be incomplete. This might indicate that MEK inhibition operates other apoptotic mechanisms independent of caspase-4 activation. Bcl-2 family members play essential roles in regulating apoptosis [31-33]. Although they are thought to function primarily on mitochondria, recent reports suggest that they can also act on the ER to which they are also located [34, 35]. Thus, inhibition of MEK might results in either inhibition of prosurvival Bcl-2 proteins such as Mcl-1 and Bcl-2 or activation of proapoptotic Bcl-2 proteins such as PUMA and Noxa as shown in other cancers [36-38].

Furthermore, the ER chaperon GRP78 seems to be a target of the MEK/ERK pathway responsible for the inhibition of ER stress-induced apoptosis. Down-regulation of GRP78 sensitized CRC cells to paclitaxel-induced apoptosis. This sensitization is caspase-dependent involving activation of caspase-3, caspase-4, and caspase-9 but not caspase-8. Using the specific inhibitors against caspase-3, caspase-4, and caspase-9 and by studying the kinetic of activation of caspases, caspase-4 activation appeared to be the initiating event. This was supported by the finding that caspase-4 specific inhibitor blocked paclitaxel-induced apoptosis. Taken together, these observations placed caspase-4 as an apical caspase in sensitization of CRC cells to paclitaxel-induced apoptosis by inhibition of MEK/ERK pathway.

How caspase-4 is activated by paclitaxel when the MEK/ERK pathway is inhibited in CRC cells is not clear. In the murine system, several mechanisms have been suggested to be responsible for ER stress-induced caspase-12 activation [4, 8]. The protease calpain, on activation by calcium released from ER, can activate caspase-12 [4]. In addition, caspase-12 has also been reported to be activated by a direct association with the ER stress transducer IRE1α and the adaptor protein TRAF2 [8]. Moreover, GRP78 was decreased and its up-regulation by ER stress was blocked by inhibition of MEK in CRC cells [20].

Co-immunoprecipitation studies revealed that GRP78 is physically associated before and after treatment with paclitaxel with caspase-4, as reported for caspase-12 in the murine system [39, 41]. These results indicate that GRP78 may participate in controlling the activation of caspase-4 in human CRC cancer cells. The physical association between GRP78 and caspase-4 also suggests that caspase-4 may be present in the ER. The amount of caspase-4 that was coprecipitated with GRP78 increased after exposure of the cells to paclitaxel. This was associated with increased amount of GRP78 in the precipitates, presumably due to increased cellular contents of the GRP78 protein induced by paclitaxel (Fig.6). The inhibitory role of GRP78 in processing of caspase-4 was confirmed by siRNA knockdown of GRP78 in that inhibition

of GRP78 increased paclitaxel-induced apoptosis.

The incomplete reverse of CRC cells resistance to paclitaxel-induced apoptosis when GRP78 is inhibited make it possible that GRP78 also protects CRC cells from ER stress-induced apoptosis by other mechanisms, such as binding to the unfolded proteins and/or calcium, thus alleviating ER stress conditions [8, 15, 42].

V. Conclusion

Taken together, these results indicate that inhibition of MEK/ERK sensitizes cultured human CRC cells to paclitaxel-induced apoptosis. This is mediated, at least in part, by activation of caspase-4, which is otherwise suppressed by the ER chaperone GRP78. These results suggest that GRP78 might be a novel mechanism underlying resistance of CRC cells to microtubule-targeting drugs by binding to and inhibition of caspase-4. Combination of compounds capable of suppressing GRP78 might be a golden approach for improving the effectiveness of taxanes in treatment of CRC.

Acknowledgment

Authors would take the opportunity to warmly thank Jordan University of Science & Technology for the financial support (grant number 225/2009).

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