The Effect of Loss of P27 and Cdk2 on Cell Cycle Progression in Response to Ultraviolet Irradiation in Mouse Embryo Fibroblasts

Eiman Aleem

Department of Zoology, Division of Molecular Biology, Faculty of Science, Alexandria University, Moharram Bey 21511, Alexandria, Egypt.
eiman.aleem@gmail.com

Abstract: It has been previously reported that ultraviolet (UV) radiation results in cell cycle arrest in the G1 phase, which may or may not be p53-dependent. Other proteins involved in DNA damage pathways induced by UV radiation include AKT and ERK. The aim of the present study was to investigate the effect of loss of cyclin-dependent kinase 2 (Cdk2) and p27 on cell cycle progression in response to UV irradiation. To achieve this goal genetically modified mouse embryo fibroblasts (MEFs) lacking Cdk2 or p27 were exposed to UV and the following parameters were investigated (1) the cell cycle kinetics of unsynchronized MEFs, (2) the cell cycle progression after first synchronization by serum starvation then UV treatment, and (3) the protein expression and activity after UV irradiation of unsynchronized MEFs. It was found that p27 and Cdk2 were required for apoptosis induced by UV. Increased expression, phosphorylation and activation of AKT in the *p27-/-* and *cdk2-/-* MEFs in comparison to wild type (WT) MEFs were observed. Similarly, the inhibitory tyrosine 15 phosphorylation of Cdk1 was increased in the *p27-/-* and *cdk2-/-* MEFs in comparison to wild type (WT) MEFs. The overall data from the present study provide clues towards understanding the role of p27 and Cdk2 in cellular response to UV radiation.
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1. Introduction

Solar ultraviolet (UV) irradiation is the most important environmental carcinogen leading to the development of skin cancer (Bode and Dong, 2003). Whereas longer UV wavelengths (UVB and UVA) induce oxidative stress and protein denaturation, short wavelength UV radiation (UVC) causes predominantly DNA damage to cells in the form of pyrimidine dimers and 6-4 photoproducts (de Gruijl *et al.*, 2001; Ravanat *et al.*, 2001). These DNA lesions are repaired enzymatically by nucleotide excision repair (Hanawalt, 2002). Due to incomplete repair, damaged bases may be misinterpreted during replication, resulting in accumulation of cancer predisposing mutations (de Gruijl *et al.*, 2001). Several DNA damage-induced signaling cascades including ATR/Chk1, jun N-terminal kinase and p38 kinase pathways are activated following UVC radiation, leading to activation of nucleotide excision repair and recovery mechanisms via transcription factors such as p53, NF-κB and AP-1 (Zhou and Elledge, 2000). These events lead to transcriptional response resulting in regulation of DNA damage repair, cell cycle progression and apoptosis. However, the molecular mechanisms underlying the cellular UV response remains to be elucidated.

It has been previously reported that UV radiation results in cell cycle arrest in the G1 phase and depending on the UV radiation dose this arrest may or may not be p53-dependent (Chang *et al.*, 1999). Other proteins involved in DNA damage pathways induced by UV radiation include AKT and ERK. It has been shown that ERK activation, after DNA damage stimuli including UV radiation, mediates cell cycle arrest and apoptosis independently of p53 (Tang et al., 2002). Progression through the G1 phase of the cell cycle is driven by cyclin-dependent kinases (Cdks), specifically Cdk4/Cdk6 and Cdk2 bound to their cyclin subunits (cyclin D and E, respectively) and the cell cycle inhibitors $p16^{INC4a}$, $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ (Sherr and Roberts, 1999). Cdks regulate not only cell cycle progression but some Cdks such as Cdk1 and Cdk2 regulate also apoptosis and DNA damage repair (Aleem and Kaldis, 2006). It was previously demonstrated that Cdk2 is required for Myc-induced apoptosis (Deb-Basu *et al.*, 2006) and for cytotoxicity and nephrotoxicity induced by cisplatin in kidney cells (Price *et al.*, 2006). In addition, Cdk2 has been demonstrated to be required for a p53-independent G2/M checkpoint control in human cells (Chung and Bunz, 2010). However, the role of Cdk2 during UV irradiation has not been elucidated. Recently, $p16^{INC4a}$ and $p27^{KIP1}$ have been suggested to be key targets in the ATR-dependent signaling pathway in response to UV damage (Al-Khalaf *et al.*, 2011). However, how p27 regulates the response to UV irradiation is not

fully understood. Therefore, the aim of the present study was to investigate the effect of loss of Cdk2 and p27 on cell cycle progression in mouse embryo fibroblasts in response to UV irradiation.

2. Materials and Methods

Cell culture

cdk2-/-, *p27-/-* and wild-type (WT) mouse embryo fibroblasts (MEFs) were generated as described in (Berthet *et al.*, 2003; Aleem *et al.*, 2005) and were maintained in culture in DMEM supplemented with 10% Fetal Bovine Serum (FBS), 1% penicillin (100 units/ml) and streptomycin (50 µg/ml) (Gibco, UK). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

UV irradiation

MEFs were seeded in 6 cm or 10 cm dishes, allowed to grow until subconfluency. Exponentially growing cultures were exposed to UVC at a dose of 50 mJ/m^2 at a Hoefer UVC 500 Ultraviolet Crosslinker (Hoefer, USA) or were mock-treated, and incubated for the indicated periods of time. Before irradiation, cells were washed with phosphate-buffered saline (PBS) and covered with 1-2 mL PBS and treated with UVC. PBS was removed, culture medium was added and cells were incubated for the indicated time points.

Cell Cycle synchronization and flow cytometry

Unsynchronized MEFs were seeded in 6 cm dishes and UV irradiated as described above, then kept in culture for 24 hrs before they were trypsinized. To synchronize cells at the G1 phase, serum starvation was performed as follows: MEFs were cultured till they were 100% confluent, then grown in DMEM supplemented with 0.1% FBS for 60 hrs before they were then UV-irradiated followed by the addition of DMEM containing 10% FBS and allowed to grow for the indicated time points to follow cell cycle progression after UV irradiation. Cells were harvested by trypsinization and collected by centrifugation and washed twice with PBS then incubated for 10 min at 37^{o}C in (0.34 mM Trisodium citrate.2 H20, 0.1% NP-40, 1.5 mM spermine tetrahydrochloride and 0.5 mM Tris (hydroxymethy1)-aminomethane containing trypsin) followed by another 10 min incubation at 37^{o}C in an equal volume of the same buffer, however, trypsin was substituted with a trypsin inhibitor. Finally, an equal volume of propidium iodide (PI) was added and cells were incubated for at least 15 min on ice in the dark. The samples were then finally analysed with a Flourescence Activated Cell Sorter FACS Calibur (Becton Dickinson), and the data were processed with the Cell Quest software (Becton Dickinson).

Preparation of lysates and Western blotting

Cells were lysed in modified RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 0.25% sodium deoxycholate). Proteases inhibitor cocktail (Roche, Germany) and phosphatase inhibitors (Sigma-aldrich, Germany) were freshly added. Lysates were centrifuged for 30 min at 16,100 x *g* at 4ºC, and supernatants were frozen at -80ºC until use. Protein concentrations were determined using the BCA protein assay (Pierce Biotechnology, Rockford, USA) according to manufacturer's protocol and concentrations measured in a microplate reader. Lysates (20 µg total protein per lane) were resolved on 12 % Bis-Tris SDS-PAGE gels. Proteins were transferred onto nitrocellulose membranes (Hybond, Amersham, UK), blocked with 5% milk in a Tris-Buffered Saline and Tween 20 (TBST) [19.97 mM Tris base, 135 mM NaCl, 0.1% Tween 20] or 5% Bovine Serum Albumin in TBST, and blotted with the following primary antibodies: mouse anti-p53 (Becton Dickenson, USA), rabbit anti-Cdk1 (Calbiochem), Cdc25C (Santa Cruz Biotechnology, USA), and the following antibodies from Cell Signaling Technology, USA: rabbit anti-phospho-Y15 Cdk1, Chk1, phosphor-Chk1 S345, AKT, pAKT T308, ERK, pERK. Membranes were routinely washed using TBST and incubated with anti-rabbit- or anti-mouse-IgG horseshoe peroxidase (HRP)-conjugated secondary antibody (Amersham, *UK*) and protein bands detected with the ECL chemiluminescence system (Amersham, UK).

3. Results

The effect of UV-irradiation on cell cycle progression of unsynchronized MEFs

In the present study unsynchronized WT, *cdk2-/-* and *p27-/-* MEFs were treated with UVC at 50 mJ/m^2 and their cell cycle analyzed after 24 hrs. Treatment of WT MEFs with UV radiation induced a 53-fold increase in the percentage of cells in sub-G1 phase (referred to in Figs. 1-4 as M1). The cells in sub-G1 represent apoptotic cells (Fig. 1A,B,G). Accordingly the percentage of cells in G1 and G2 phases decreased by 2.6 and 2.3 fold, respectively, in comparison to the untreated WT MEFs. On the other hand, the percentage of cells in S phase increased by about 1.8 fold (Fig. 1A,B,G). In contrast, the loss of p27 or Cdk2 had a protective effect against apoptosis induced by UV in unsynchronized MEFs because the percentage of cells in sub-G1 was much less in comparison to that in WT MEFs. Treatment of *p27-/-* (Fig. 1C,D,G) or *cdk2-/-* MEFs (Fig. 1 E,F,G) with UV induced only 11.4 fold increase in the percentage of cells in sub-G1 phase relative to the corresponding untreated cells. This indicates that p27 and Cdk2 are required for apoptosis induced by UV irradiation in unsynchronized MEFs. UV treatment also induced a

decrease in both G1 and G2/M phases of the cell cycle in *p27-/-* and *cdk2-/-* MEFs. The most resistant cell line to cell cycle perturbations induced by UV irradiation was the *cdk2-/-* MEFs (Fig. 1 E,F,G) .

Effect of loss of p27 and Cdk2 on cell cycle progression after synchronization at G1 phase and UV irradiation

Next I asked the question whether the loss of p27 and Cdk2 would alter the ability of cells to progress through the cell cycle after UV irradiation. In order to follow the cell cycle kinetics cells must be synchronized first at a certain phase then treated with UV and monitored after different time points. In the present study, WT-, *p27-/-* and *cdk2-/-* MEFs were synchronized by serum starvation for 60 hrs. This time point is referred to as zero h (0 h) in (Figs. 2,3,4). Thereafter, cells were either treated with UV then immediately replaced in DMEM with 10% FBS (regular growth conditions) or replaced directly in DMEM supplemented with 10% FBS and used as control. The cells were then allowed to grow in normal growth conditions and then harvested after 12- and 24 hrs, respectively. When WT MEFs were serum starved for 60 hrs there was about 15-fold increase in the sub-G1 cell population (apoptotic fraction) in comparison to unsynchronized WT MEFs (Fig. 2F versus Fig. 1G). In addition, the percentage of cells in S phase dropped by serum starvation to about one fifth the value in unsynchronized WT MEFs, whereas the percentage of cells in the G1 phase did not change much (Fig. 2A, F).

Following up the cell cycle progression in control WT MEFs showed that the percentage of cells in S phase gradually increased at the 12- (Fig. 2B) to the 24 hrs time points (Fig. 2D) indicating that cells normally progressed into S phase after being returned to normal growth conditions and also the percentage of cells in G2/M phase increased at the 24 hr time point to reach about 2.7-fold the corresponding value at 0 hr indicating that cells successfully entered the G2/M phase after 24 hrs from re-feeding (Fig. 2 D, F). The effect of UV irradiation on serum starved WT MEFs was a gradual increase in the sub-G1 phase population from 12 hrs to reach 26-fold the corresponding value of WT MEFs control at the same time point. Similarly, the percentage of aneuploid cells (cells with abnormal DNA content) in WT MEFs increased by UV irradiation (Fig. 2 C,E,F). Therefore, in synchronized WT MEFs UV irradiation caused death by apoptosis rather than cell cycle arrest. In *p27-/-* MEFs, serum starvation for 60 hrs resulted in an increase in the percentage of apoptotic and aneuploid cells (Fig. 3A,F) in comparison to unsynchronized MEFs, however, the cell cycle progressed normally after serum re-feeding as indicated by the increase in the S and G2/M phase populations (Fig. 3 A,B,D).

In contrast to WT MEFs, UV irradiation of serum starved *p27-/-* MEFs resulted in an initial G1 phase arrest which was obvious after 12 hrs (Fig. 3 B,C, F), followed by an increase in apoptotic cells (sub-G1 fraction) after 24 hrs in comparison to the control *p27-/-* MEFs at the same time points (Fig. 3 B,C, D,E,F). Serum starvation of *cdk2-/-* MEFs for 60 hrs resulted in a G2/M phase arrest instead of the expected G1 arrest. Comparison of the cell cycle profiles of unsynchronized (Fig. 1E) and serum starved *cdk2-/-* MEFs at the (0 h) (Fig. 4A) revealed that the percentage of cells in the G2/M phase increased from 45 to 71 % while the percentage of cells in the G1 phase decreased from 25 to 15%. Following the cell cycle kinetics of *cdk2-/-* MEFs after serum re-feeding revealed that cells did not progress in the cell cycle (Fig. 4B,F), instead the percentage of cells with abnormal DNA content (aneuploid) increased after 24 hrs to reach 46% compared to 8% at the (0h) (Fig. 4 D,F). The cell cycle profiles of UV-irradiated *cdk2-/-* MEFs after 12 hrs was very similar to the control *cdk2-/-* MEFs (Fig. 4 B,C). However, surprisingly after 24 hrs the UV-irradiated *cdk2-/-* MEFs had a similar to "normal" cell cycle profile with 54% of the cells in G2/M and 22 % in G1 phase of, and 10% in the sub-G1 phase (apoptotic cells) of the cell cycle (Fig. 4E,F). This indicated that the loss of Cdk2 protected MEFs from DNA damage accumulated as a result of serum starvation or UV irradiation.

Effect of UV irradiation on protein expression and phosphorylation in p27 and Cdk2-deficient mouse embryo fibroblasts

First a Western blot using antibodies against p27 and Cdk2 was performed to confirm the genotype of MEFs used in the present study (Fig. 5A). Only WT- and *cdk2-/-* MEFs expressed p27 (Fig. 5A, upper panel), while only WT- and *p27-/-* MEFs expressed Cdk2 (Fig. 5A, lower panel). To investigate the effect of UV irradiation on protein expression and activation, unsynchronized MEFs were treated with UV then allowed to grow in normal growth conditions for 24 hrs before harvesting. The survival proteins AKT and ERK (Fig. 5B) and the checkpoint pathway proteins; Chk1, Cdc25C and Cdk1 were studied (Fig. 5C). In the present study UV irradiation induced the phosphorylation of AKT at T308 in WT-, as well as *p27-/-* and *cdk2-/-* MEFs, however, the phosphorylation was more pronounced in *p27-/-* and *cdk2-/-* MEFs in comparison to WT MEFs (Fig. 5B, top panel) in spite of the fact that the total protein in the UV-treated MEFs was almost not detected (Fig. 5B, second panel from top).

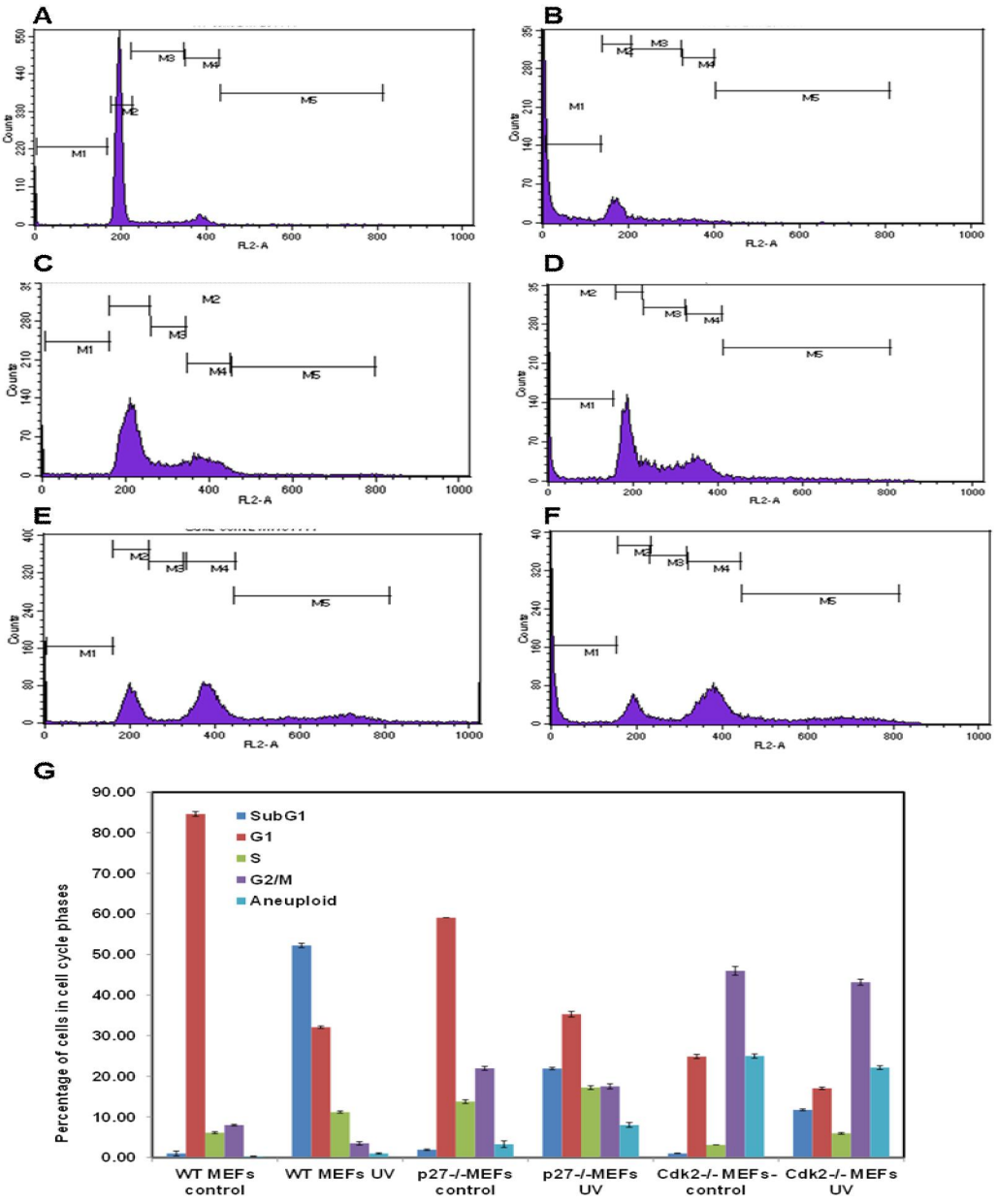
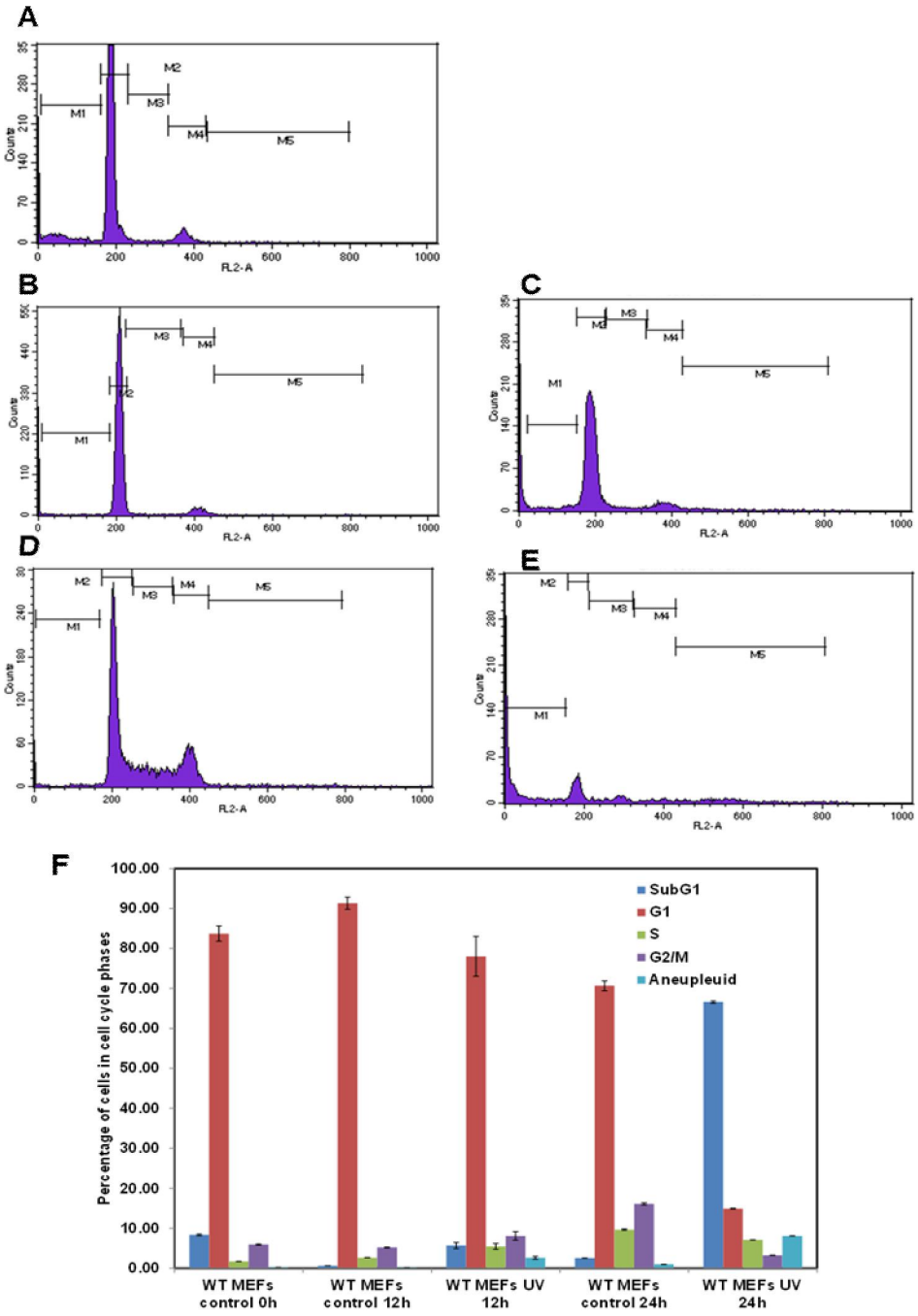


Figure 1. FACS analysis showing the effect of UV irradiation on cell cycle progression in unsynchronized mouse embryo fibroblasts deficient for p27 and Cdk2 in comparison to wild type MEFs (WT). MEFs were treated with UV then allowed to grow for 24 hrs before harvesting. (A) WT MEFs control, (B) WT MEFs treated with UV, (C) *p27-/-* MEFs control, (D) *p27-/-* MEFs treated with UV, (E) *cdk2-/-* MEFs control, (F) *cdk2-/-* MEFs treated with UV, (G) Histogram showing the percentage of cells in different cell cycle phases (y-axis) against the different cell lines (x-axis).



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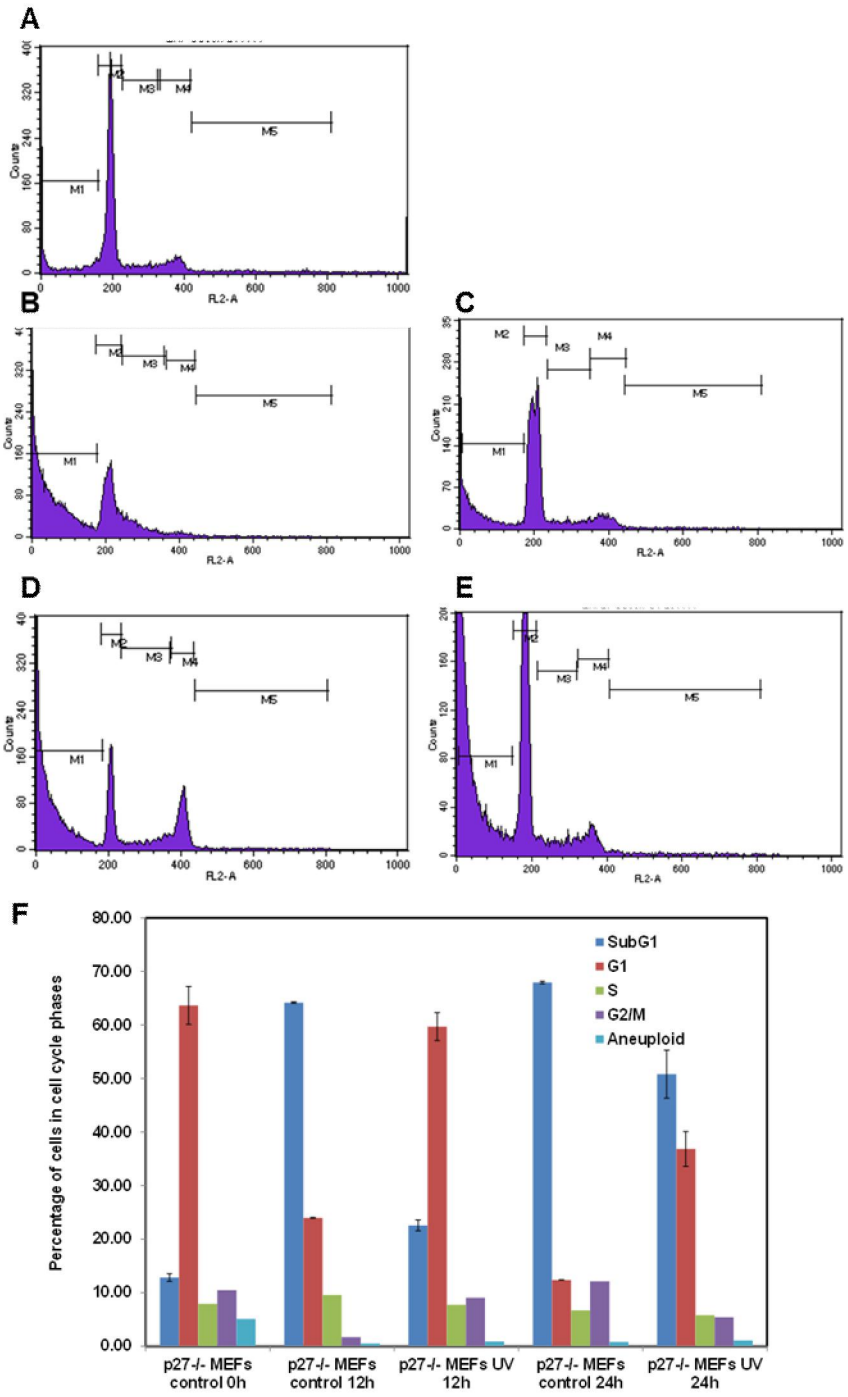

Figure 3. FACS analysis showing the effect of UV irradiation on cell cycle progression in synchronized *p27-/-* mouse embryo fibroblasts. *p27-/-* MEFs were serum starved for 60 hrs (0 h time point), treated with UV then allowed to grow in normal growth conditions for 24 hrs before harvesting. (A) *p27-/-* MEFs at the zero h time point (0 h), (B) control *p27-/-* MEFs at 12 hrs after re-feeding, (C) *p27-/-* MEFs treated with UV after 12 hrs of re-feeding, (D) control *p27-/-* MEFs at 24 hrs after re-feeding, (E) *p27-/-* MEFs treated with UV after 24 hrs of re-feeding. (F) Histogram showing the percentage of cells in different cell cycle phases (y-axis) against the different cell lines (x-axis).

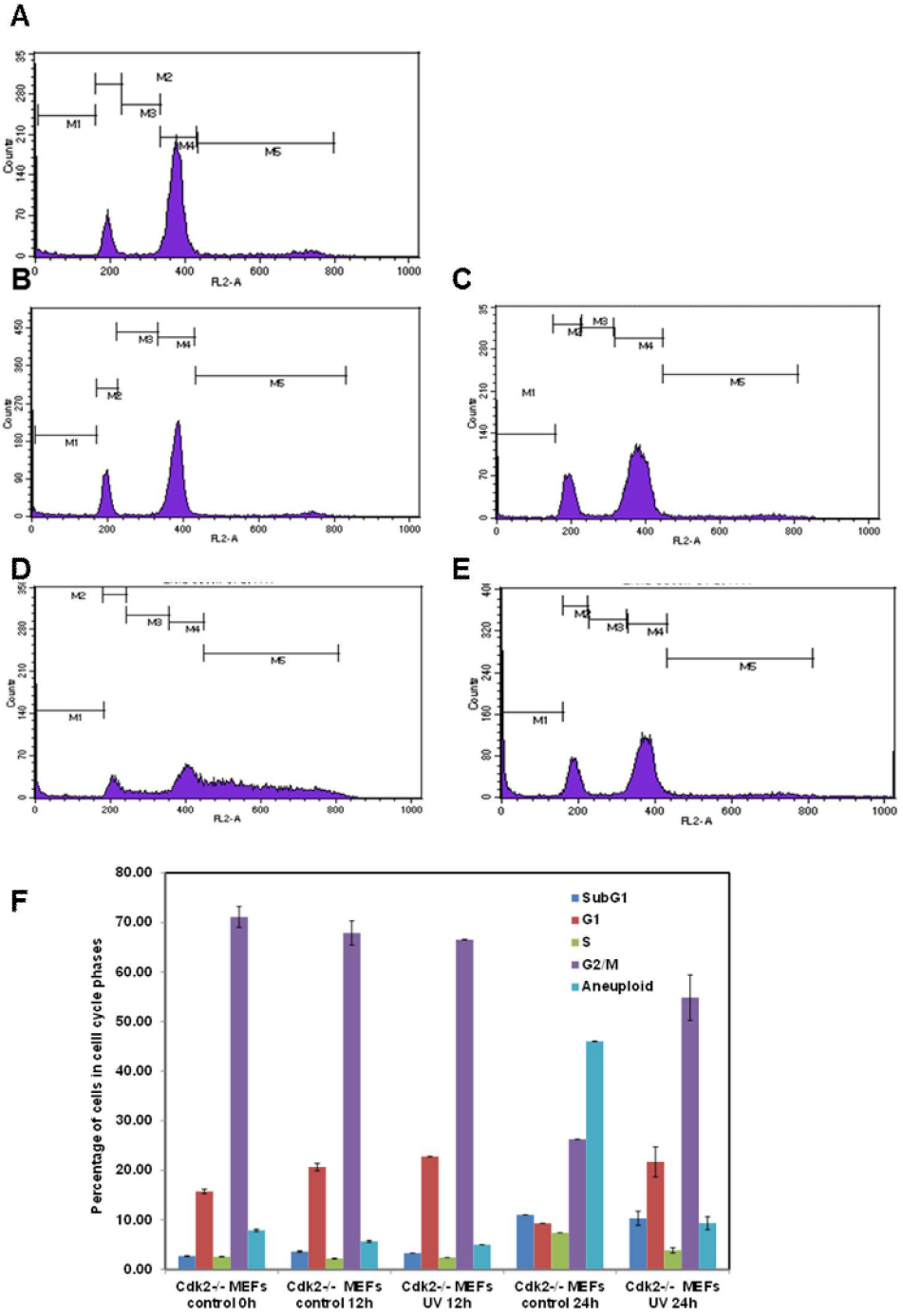

Figure 4. FACS analysis showing the effect of UV irradiation on cell cycle progression in synchronized *cdk2-/-* mouse embryo fibroblasts. *Cdk2-/-* MEFs were serum starved for 60 hrs (0 h time point), treated with UV then allowed to grow in normal growth conditions for 24 hrs before harvesting. (A) *cdk2-/-* MEFs at the zero h time point (0 h), (B) control *cdk2-/-* MEFs at 12 hrs after re-feeding, (C) *cdk2-/-* MEFs treated with UV after 12 hrs of re-feeding, (D) control *cdk2-/-* MEFs at 24 hrs after re-feeding, (E) *cdk2-/-* MEFs treated with UV after 24 hrs of re-feeding. (F) Histogram showing the percentage of cells in different cell cycle phases (y-axis) against the different cell lines (x-axis).

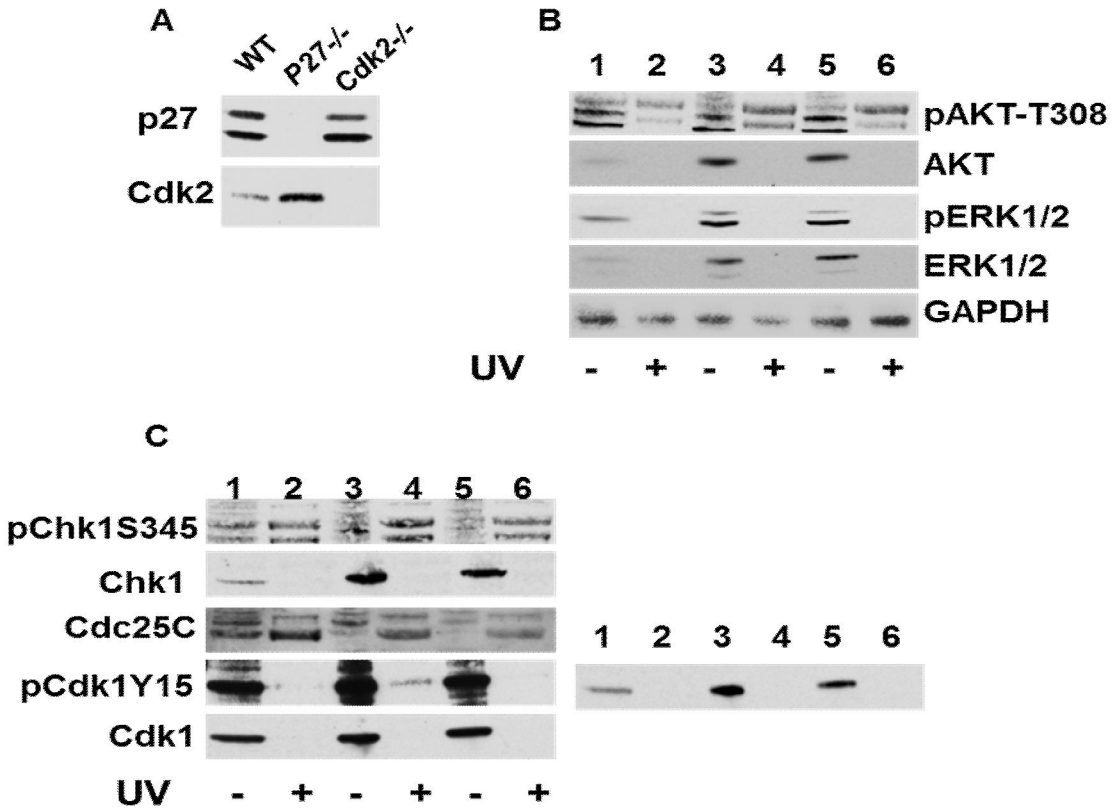

Figure 5. Western blot showing the effect of UV irradiation on protein expression and phosphorylation (A) Samples from WT-, *p27-/-* and *cdk2-/-* MEFs to show the genotype of each cell line. (B) Activation and protein levels of AKT and ERK. (C) Left panels show the activation and protein levels of Chk1, Cdc25C, Cdk1 and the right panel shows lower exposure of pCdk1Y15 for better illustration of its differential expression in the three genotypes.

AKT was overexpressed in untreated *p27-/-* and *cdk2-/-* MEFs in comparison to untreated WT MEFs (Fig. 5B, second panel from top). Similarly, ERK1/2 and their phosphorylated forms were overexpressed in the untreated *p27-/-* and *cdk2-/-* MEFs in comparison to untreated WT MEFs, but were not detected after UV irradiation (Fig. 5B, third and fourth panels from top). The Chk1 phosphorylation at S345 indicating its activation was detected in all the three genotypes after UV irradiation and the protein levels of Chk1 were overexpressed in the untreated *p27-/-* and *cdk2-/-* MEFs in comparison to untreated WT MEFs (Fig. 5C, first and second panels from top). The downstream target of Chk1, Cdc25C, was overexpressed after UV irradiation in the three genotypes (Fig. 5C, third panel from top). Cdk1 and its inhibitory site tyrosine 15 (Y15) were overexpressed also in the untreated *p27-/-* and *cdk2-/-* MEFs in comparison to untreated WT MEFs (Fig. 5C, fourth and fifth panels from top), and was overexpressed after UV irradiation in the *p27-/-* MEFs in comparison to both UV-treated WT and *cdk2-/-* MEFs (Fig. 5C, fourth panel from top).

4. Discussion

In the present study the effect of loss of p27 and Cdk2 on cellular response to UV irradiation in mouse embryo fibroblasts (MEFs) was studied. The genetically modified MEFs were previously generated using gene targeting by (Berthet et al., 2003; Aleem et al., 2005). Three parameters were investigated in the present study (1) The effect of UV irradiation on unsynchronized MEFs, (2) The effect of UV irradiation on cell cycle progression after synchronization by serum starvation, and (3) protein expression and activity after UV irradiation of unsynchronized MEFs. It was demonstrated that unsynchronized wild-type MEFs (WT) were more sensitive to UV irradiation than both *p27-/-* and *cdk2-/-* MEFs because a larger percentage of apoptosis was detected in WT MEFs than in the other two genotypes after irradiation. In order to understand the molecular mechanism underlying this differential response to UV-induced apoptosis the protein levels and activation of AKT, ERK, and proteins involved in DNA damage repair pathway were studied. In general, UV treatment induced

downregulation in the total protein levels in the three genotypes (WT-, *p27-/-* and *cdk2-/-* MEFs). This may be attributed to a general downregulation in the protein translation machinery; however the small amount of remaining proteins retained their ability to be phosphorylated and activated by UV.

In the present study, phosphorylation of AKT at T308 was increased by UV irradiation in *p27-/-* and *cdk2-/-* MEFs more than in WT MEFs. These results are in agreement with previous findings by Huang et al. (2001) who demonstrated that UV induces AKT phosphorylation at both Ser-473 and Thr-308. It is noted in the present study that AKT is overexpressed in the *p27-/-* and *cdk2-/-* MEFs even without UV irradiation in comparison to WT MEFs. AKT is a survival factor. Akt activity is regulated by phosphorylation on four phosphorylation sites identified including Ser-124, Thr-450, Thr-308, and Ser-473 (Alessi *et al.*, 1996; Datta *et al.*, 1999). Mutagenesis studies have suggested that the phosphorylation of Thr-308 and Ser-473 is required for Akt activity, whereas Ser-124 and Thr-450 appear to be basally phosphorylated (Alessi *et al.*, 1996; Datta *et al.*, 1999). Taken together, increased phosphorylation on T308 of AKT in *p27-/-* and *cdk2-/-* MEFs indicate that AKT is activated upon UV irradiation in these MEFs and this may explain the reduced apoptosis in these cells in comparison to WT MEFs. In agreement with this explanation it has been reported before that the transfection of a variety of cell types with constitutively active AKT blocks apoptosis induced by apoptotic stimuli such as UV radiation, DNA damage, and the anti-fas antibody (Datta *et al.*, 1999).

In the present study total protein and phosphorylated protein levels of Chk1, Cdc25C and Cdk1 and its inhibitory phosphorylation site Y15 were studied. Similar to AKT, Chk1 was phosphorylated on S345 in WT-, *p27-/-* and *cdk2-/-* MEFs upon UV irradiation and Chk1 protein levels were overexpressed in the untreated *p27-/-* and *cdk2-/-* in comparison to WT MEFs. Chk1 is a critical transducer of signals that arise at exogenously induced DNA strand breaks but it is also required for normal cell growth (Liu *et al.*, 2000). As a checkpoint protein, after DNA damage, Chk1 is phosphorylated upon multiple residues, including S317 and S345 (Liu *et al.*, 2000; Zhao and Piwnica-Worms, 2001). Chk1 is then released from chromatin and accumulates at the centrosome, where it is thought to prevent activation of Cdk1 and entry into mitosis (Loffler *et al.*, 2006; Smits, 2006). The obtained results are in agreement with this notion because in the present study phosphorylation on S345 of Chk1 was most increased in the *p27-/-* MEFs upon UV irradiation in comparison to WT and *cdk2-/-* MEFs and accordingly the phosphorylation of Cdk1 at the inhibitory site Y15 was also most pronounced in the *p27-/-* MEFs after UV irradiation in comparison to the other two genotypes indicating that Cdk1 is inhibited in this cell line after UV treatment. In addition, phosphorylation at this inhibitory site was increased in the untreated *p27-/-* and *cdk2-/-* MEFs than in the WT MEFs. This may explain the accumulation of cells in the G1 phase in the untreated *p27-/-* MEFs and the accumulation of cells in G2 phase in the untreated *cdk2-/-* MEFs since Cdk1 regulates both G1 and G2/M phase transition (Aleem et al., 2005). Therefore, if Cdk1 is retained in the inhibited state as is the case in the untreated *p27-/-* and *cdk2-/-* MEFs cells may accumulate in G1 or G2/M phase as is the case in the present study. A downstream target of Chk1 is the Cdc25C, which was overexpressed in the UV-treated WT MEFs than the *p27-/-* and *cdk2/-* MEFs. Since Cdc25C is a positive regulator of Cdk1; it acts by dephosphorylating the inhibitory tyrosine 15, thus rendering Cdk1 fully active, this may explain why the Y15 phosphorylation was not detected in WT MEFs upon UV treatment in comparison to *p27-/-* or *cdk2-/-* MEFs.

The present study suggests a potential involvement of p27 in mediating UV damage signal. This is consistent with a very recent report by Al-Khalaf et al. (2011) who demonstrated that p16 and p27 are key targets for ATR-dependent signaling pathway in response to UV irradiation. It is known that p27 can act both as a tumor suppressor gene and as an oncogene (Besson *et al.*, 2004), therefore, results from the present study indicate that p27 is important for the process of apoptosis induced by. Similarly, the fact that the percentage of apoptosis is reduced in *cdk2-/-* MEFs upon UV irradiation, in addition to the accumulation of cells in G2/M phase reflects the requirement of Cdk2 in the process of apoptosis induced by UV. This is in consistence with our previous results that Cdk2 is required for Myc-induced apoptosis and for cisplatin-induced toxicity (Deb-Basu et al., 2006; Price et al., 2006). Therefore, Cdk2 may play a role in the regulation of apoptosis regardless of the apoptotic cause whether it is Myc, cisplatin or UV. In the present study, UV treatment of cells synchronized by serum starvation did not inhibit cell cycle progression in any of the three genotypes, however, there was a differential response in each genotype. Whereas UV after serum starvation induced a high percentage of apoptosis in the WT-MEFs, it initiated a G1 arrest and a G2/M arrest in the *p27-/-* and *cdk2-/-* MEFs, respectively.

Taken together, the results of the present study

highlight the importance of both p27 and Cdk2 as modifiers of UV radiation response in mouse embryo fibroblasts.

Corresponding author
Eiman Aleem
Department of Zoology, Division of Molecular Biology, Faculty of Science, Alexandria University, Moharram Bey 21511, Alexandria, Egypt.
eiman.aleem@gmail.com

References

Al-Khalaf, H. H., Hendrayani, S. F., and Aboussekhra, A. (2011). The atr protein kinase controls UV-dependent upregulation of p16INK4A through inhibition of Skp2-related polyubiquitination/degradation. *Molecular cancer research : MCR* **9**, 311-319.

Aleem, E., and Kaldis, P. (2006). Mouse models of cell cycle regulators: new paradigms. *Results and problems in cell differentiation* **42**, 271-328.

Aleem, E., Kiyokawa, H., and Kaldis, P. (2005). Cdc2-cyclin E complexes regulate the G1/S phase transition. *Nature cell biology* **7**, 831-836.

Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., and Hemmings, B. A. (1996). Mechanism of activation of protein kinase B by insulin and IGF-1. *The EMBO journal* **15**, 6541-6551.

Berthet, C., Aleem, E., Coppola, V., Tessarollo, L., and Kaldis, P. (2003). Cdk2 knockout mice are viable. *Current biology* **13**, 1775-1785.

Besson, A., Gurian-West, M., Schmidt, A., Hall, A., and Roberts, J. M. (2004). p27Kip1 modulates cell migration through the regulation of RhoA activation. *Genes & development* **18**, 862-876.

Bode, A. M., and Dong, Z. (2003). Mitogen-activated protein kinase activation in UV-induced signal transduction. *Science's STKE* **2003** (167), RE2.

Chang, D., Chen, F., Zhang, F., McKay, B. C., and Ljungman, M. (1999). Dose-dependent effects of DNA-damaging agents on p53-mediated cell cycle arrest. *Cell growth & differentiation* **10**, 155-162.

Chung, J. H., and Bunz, F. (2010). Cdk2 is required for p53-independent G2/M checkpoint control. *PLoS genetics* **6**, e1000863.

Datta, S. R., Brunet, A., and Greenberg, M. E. (1999). Cellular survival: a play in three Akts. *Genes & development* **13**, 2905-2927.

de Gruijl, F. R., van Kranen, H. J., and Mullenders, L. H. (2001). UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. *Journal of photochemistry and photobiology* **63**, 19-27.

Deb-Basu, D., Aleem, E., Kaldis, P., and Felsher, D. W. (2006). CDK2 is required by MYC to induce apoptosis. *Cell Cycle* **5**, 1342-1347.

Hanawalt, P. C. (2002). Subpathways of nucleotide excision repair and their regulation. *Oncogene* **21**, 8949-8956.

Huang, C., Li, J., Ding, M., Leonard, S.S., Wang, L., Castranova, V., Vallyathan, V. and Shi, X. (2001). UV induces phosphorylation of protein kinase B (Akt) at Ser-573 and Thr-308 in mouse epidermal Cl 41 cells through hydrogen peroxide. *The Journal of Biological Chemistry* **276**, 40234-40240

Liu, Q., Guntuku, S., Cui, X. S., Matsuoka, S., Cortez, D., Tamai, K., Luo, G., Carattini-Rivera, S., DeMayo, F., Bradley, A., Donehower, L. A., and Elledge, S. J. (2000). Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. *Genes & development* **14**, 1448-1459.

Loffler, H., Lukas, J., Bartek, J., and Kramer, A. (2006). Structure meets function--centrosomes, genome maintenance and the DNA damage response. *Experimental cell research* **312**, 2633-2640.

Price, P. M., Yu, F., Kaldis, P., Aleem, E., Nowak, G., Safirstein, R. L., and Megyesi, J. (2006). Dependence of cisplatin-induced cell death in vitro and in vivo on cyclin-dependent kinase 2. *Journal of the American Society of Nephrology : JASN* **17**, 2434-2442.

Ravanat, J. L., Douki, T., and Cadet, J. (2001). Direct and indirect effects of UV radiation on DNA and its components. *Journal of photochemistry and photobiology* **63**, 88-102.

Sherr, C. J., and Roberts, J. M. (1999). CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes & development* **13**, 1501-1512.

Smits, V. A. (2006). Spreading the signal: dissociation of Chk1 from chromatin. *Cell Cycle* **5**, 1039-1043.

Tang, D., Wu, D., Hirao, A., Lahti, J.M., Liu, L., Mazza, B., Kidd, V.J., Mak, T.W. and Ingram, A.J. (2002). ERK activation mediates cell cycle arrest and apoptosis after DNA damage independently of p53. *The journal of Biological Chemistry* **277**, 12710-12717.

Zhao, H., and Piwnica-Worms, H. (2001). ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. *Molecular and cellular biology* **21**, 4129-4139.

Zhou, B. B., and Elledge, S. J. (2000). The DNA damage response: putting checkpoints in perspective. *Nature* **408**, 433-439.

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