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Activation of PKR/eIF2α Signaling Cascade is Associated With Dihydrotestosterone-Induced Cell Cycle Arrest and Apoptosis in Human Liver Cells

Rongyang Dai,[1*] Dongmei Yan,[1] Juan Li,[1] Shaokun Chen,[2] Youping Liu,[1] Run Chen,[2] Chunyan Duan,[1] Mei Wei,[3] Hong Li,[1] and Tao He[1*]

[1]*Department of Biochemistry of Luzhou Medical College, Luzhou, Sichuan, China*
[2]*Department of Public Health of Luzhou Medical College, Luzhou, Sichuan, China*
[3]*The Affiliated Hospital of Chinese Traditional Medicine of Luzhou Medical College, Luzhou, Sichuan, China*

ABSTRACT

Androgen receptor (AR) signaling plays an important role in the development and progression of several liver diseases, including hepatocellular carcinoma (HCC) and non-alcoholic fatty liver disease (NAFLD). Dihydrotestosterone (DHT) is the active metabolite of the major circulating androgen, testosterone. In this study, we investigated the effect of DHT on human liver cells. We found that DHT not only induces cell cycle arrest but also initiates apoptosis in androgen-sensitive liver cells, such as SMMC-7721 and L02. Importantly, DHT/AR induces the activation of RNA-dependent protein kinase (PKR)/eukaryotic initiation factor-2 alpha (eIF2α) cascades in androgen-sensitive liver cells. PKR/eIF2α activation-induced growth arrest and DNA damage-inducible gene 153 (GADD153) and heat shock protein 27 (Hsp27) expression contribute to cell cycle arrest in response to DHT. It is notable that DHT administration results in androgen-sensitive liver cells apoptosis, at least in part, through PKR/eIF2α/GADD153 cascades. These results suggest that the androgen/AR pathway plays a pivotal role in liver cell growth and apoptosis regulating, whose deregulation might be involved in the pathogenesis of liver diseases. J. Cell. Biochem. 113: 1800–1808, 2012.

KEY WORDS: DIHYDROTESTOSTERONE; CELL CYCLE ARREST; APOPTOSIS; LIVER CELLS; PKR/eIF2α

The steroid hormone androgen is essential for male sexual development and maintenance of the male phenotypes [Kokontis and Liao, 1999]. The reduced form of androgen, which is converted by the specific 5-α-reductase, is active and exerts its biological functions via androgen receptor (AR) in androgen-responsive tissues or organs [Anderson and Liao, 1968; Bruchovsky and Wilson, 1968; Liao, 1994]. Dihydrotestosterone (DHT), which has an essential role in androgen/AR signaling, is a biologically active metabolite of testosterone formed by the enzyme 5-α-reductase by means of reducing the Δ4,5 double-bond [Bartsch et al., 2002]. AR is a member of the steroid hormone receptor superfamily. In the absence of androgen engagement, AR remains in the cytoplasm [Roberts et al., 1978; Georget et al., 1997]. Upon binding to androgen, the androgen/AR complex translocates into the nucleus and induces the expression of androgen target genes that are involved in many cellular activities, such as proliferation and apoptosis [Anderson and Liao, 1968; Bruchovsky and Wilson, 1968; Kokontis et al., 1998; Kokontis and Liao, 1999].

Regulation of gene expression at the translational level plays critical roles in cell proliferation and apoptosis [Holcik and Sonenberg, 2005]. Translation can be controlled at each of the three steps: Initiation, elongation, and termination [Hershey, 1991]. However, most of translational control is known to be exerted primarily at the level of initiation [Dever, 2002]. A critical event of initiation regulation is through the regulation of translation initiation factor eukaryotic initiation factor-2 alpha (eIF2α) activity. The phosphorylation on S51 of eIF2α results in blocking of protein synthesis initiation through inhibiting the guanine nucleotide

Conflicts of interest: None.

Rongyang Dai, Dongmei Yan, and Juan Li contributed equally to this work.

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*Correspondence to: Rongyang Dai or Tao He, Department of Biochemistry, Luzhou Medical College, 319 Zhong Shan Road, Luzhou 646000, Sichuan, China. E-mail: dryrun2502@163.com; hetao198@163.com

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exchange factor [Dever, 2002]. Phosphorylation of eIF2α is mediated by four kinases, RNA-dependent protein kinase (PKR), protein kinase RNA (PKR)-like ER kinase (PERK), general control non-derepressible-2 (GCN2), and heme-regulated kinase (HRI) under distinct forms of stress conditions [Chen and London, 1995; Harding et al., 2000; Dever, 2002; Dey et al., 2007; Raven and Koromilas, 2008]. Among them, PKR is the most widely studied member. It has been well known that the activation of PKR leads to cell proliferation inhibition and destruction of cells by apoptosis [Jagus et al., 1999].

Activating transcription factor 4 (ATF4) is the key target downstream of eIF2α phosphorylation. Translation of ATF4 is repressed by the presence of two upstream open reading frames (uORFs) [Vattem and Wek, 2004]. Upon eIF2α phosphorylation, ribosomes scan initiates ATF4 translation through the uORFs [Vattem and Wek, 2004]. ATF4 induces genes responsible for the antioxidant response, amino acid metabolism, and apoptosis [Cherasse et al., 2007; Malhi and Kaufman, 2011; Xu et al., 2005]. Growth arrest and DNA damage-inducible gene 153 (GADD153) is a key apoptosis inducer downstream of ATF4. Possible mediators of GADD153-induced apoptosis include BCL2, growth arrest- and DNA damage-inducible gene 34 (GADD34), endoplasmic reticulum oxidoreductin 1 (ERO1α) and tribbles-related protein 3 (TRB3) [Szegezdi et al., 2006].

Recently, it has been reported that androgen/AR signaling plays a critical role in the development of hepatocellular carcinoma (HCC) and non-alcoholic fatty liver disease (NAFLD) [Jie et al., 2007; Lin et al., 2008; Wu et al., 2010]. Here, we report that PKR/eIF2α activation is involved in androgen/AR-induced cell cycle arrest and apoptosis in androgen-sensitive liver cells.

MATERIALS AND METHODS

MATERIALS

PKR inhibitor C16, thapsigargin (Tg), tunicamycin (Tm), DHT, and flutamide (Flu) were purchased from Sigma Chemical Company (St. Louis, MO). GADD153 siRNA, PKR siRNA, Hsp27 siRNA, and the antibodies against AR, GRP78, GADD153, Hsp27, and eIF2α were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against PARP, phospho-Akt (Ser473), Akt, and phospho-eIF2α (Ser51) were purchased from Cell Signaling Technology (Beverly, MA). Antibody against GAPDH was purchased from KangChen Bio-tech Incorporation (Shanghai, China).

CELL CULTURE AND TREATMENT

Human liver cell lines HepG2, SMMC-7721, and L02 were maintained at 37°C in a humidified incubator containing 5% CO_2, in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. RAW 264.7 macrophages were maintained in RPMI 1640 medium. Tg and Tm were used to induce the ER stress response. To maintain the required concentration of DHT, the medium was replaced every 12 h. The protocol used for GADD153, Hsp27, and PKR knockdown is as described previously [Pyrko et al., 2007; Dai et al., 2009].

RNA PREPARATION AND RT-PCR ANALYSIS

Total RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The reverse transcription reactions were carried out using the M-MLV Reverse Transcriptase (Promega) according to the manufacturer's protocol. PCR products were resolved by electrophoresis in a 2 or 4% agarose gel, stained with ethidium bromide. The gel images were digitally captured with a SynGene gel documentation system and analyzed with the Genetools analysis software (Syngene, Frederick, MD). All tests were repeated thrice, and one of the repeats was shown in the results. The primers used in this study are shown in Table S1.

WESTERN BLOT ANALYSIS

Cells were lysed in Triton lysis buffer (20 mM Tris, pH 7.4, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, 1 mM PMSF, 10 mM NaF, 5 mg/ml aprotinin, 20 mM leupeptin, and 1 mM sodium orthovanadate) and centrifuged at 12 000*g* for 15 min. Protein concentrations were measured using the BCA assay (Santa Cruz Biotech, CA). Equal proteins were applied to SDS–PAGE. After electrophoresis, proteins were blotted to polyvinylidene difluoride (PVDF) membranes and then blocked with 5% skim milk powder with 0.1% Tween-20. The blots were then probed at 4 °C overnight with the relevant primary antibodies, washed three times with TBST (TBS containing 0.1% Tween-20), and probed with the appropriate horseradish-peroxidase-conjugated secondary antibodies at room temperature for 2 h. Immunoreactive material was detected using the ECL kit (Santa Cruz, CA) according to the manufacturer's instruction. The intensities of the bands were densitometrically quantified.

CELL CYCLE ANALYSIS

Cells growing exponentially in 6-well plates were synchronized (G_0/G_1) by placing in serum-free DMEM for 20 h before treatment with DHT for the indicated time. After fixed with 70% ethanol at 4 °C overnight, the cells were washed with PBS and stained with 0.05 mg/ml PI, which stains DNA, containing 0.2 mg/ml RNase for 30 min at 37 °C. The fluorescence volume of PI was measured by flow cytometry, and the percentage of cells in each phase of the cell cycle was calculated. The experiments were repeated three times.

APOPTOSIS ANALYSIS

Apoptosis was detected using Annexin V-FITC Apoptosis Detection Kit (Invitrogen) according to the manufacturer's manual. Annexin V staining was analyzed by flow cytometry within 1 h. The experiments were repeated thrice.

STATISTICAL ANALYSIS

Results are expressed as mean ± standard deviation. Statistical analysis was performed using Student's *t*-test. *P*-values lower than 0.05 was considered statistically significant.

RESULTS

DHT INDUCES CELL CYCLE ARREST

First, mouse macrophage cell line RAW264.7 and human liver cell lines, L02, SMMC-7721, and HepG2, were utilized to detect the

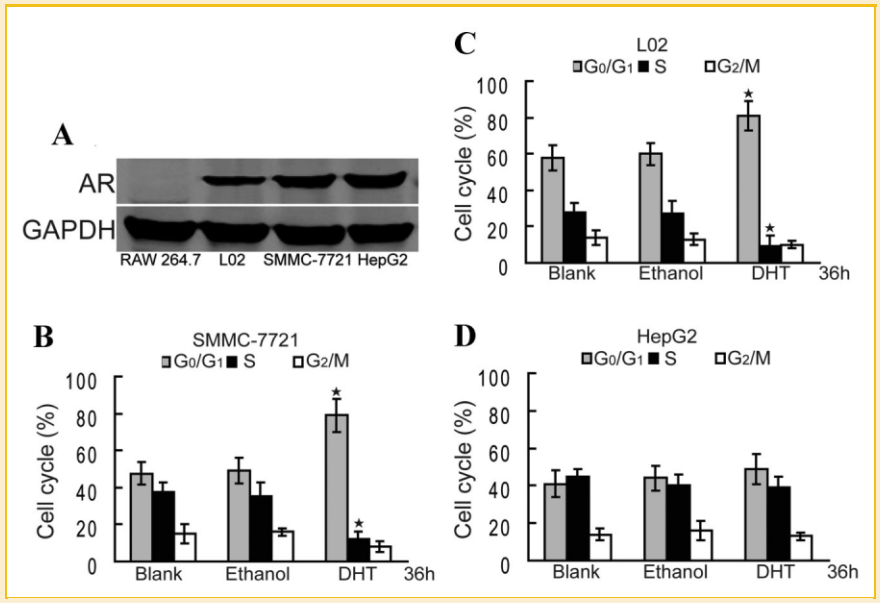

Fig. 1. DHT induces cell cycle arrest. A: Western blot analysis of protein levels of AR in various human liver cell lines. RAW 264.7 cells were used as negative control. B–D: The effect of DHT on cell cycle progress in human liver cell lines. After treated with DHT (100 nM) for 36 h, SMMC-7721 (B), L02 (C), and HepG2 (D) cells were subjected to measurement of cell cycle distribution using flow cytometry. Data are presented as mean values ± SD of three measurements. Columns, mean of three individual experiments; bars, SE. *Significantly different from control value.

protein levels of AR. As shown in Figure 1A, L02, SMMC-7721, and HepG2 cells show strong expression of AR compared to RAW264.7 cells. To study the effect of androgen on the cell cycle progress in liver cells, L02, SMMC-7721, and HepG2 cells were incubated with DHT for indicated time periods. The effect of DHT on the cell cycle progress was detected by flow cytometry. DHT incubation resulted in reduction in the fraction of S phase cells, with a concomitant increase in the percentage of G_0/G_1 phase cells in SMMC-7721 and L02 cells (Fig. 1B,C). In contrast, DHT had no effect on the cell cycle progress in HepG2 (Fig. 1D) and RAW264.7 (data not shown) cells. It has been reported that DHT promotes prostate cancer cells growth at low doses [Tsihlias et al., 2000], but we found that DHT had no apparent effect on the cell cycle progress in SMMC-7721 and L02 cells at concentrations below 10 nM (Supplementary Fig. 1). These data suggest that DHT induces cell cycle arrest in some AR-positive liver cell lines in a concentration-dependent manner.

GADD153 AND HSP27 ARE INVOLVED IN DHT-INDUCED CELL CYCLE ARREST

Previous studies demonstrated that androgen induces PI3K/Akt activation in androgen-sensitive epithelial cells [Sun et al., 2003; Baron et al., 2004]. We tested the effect of DHT on the PI3K/Akt pathway in human liver cells. SMMC-7721, L02, and HepG2 cells were cultured in media containing DHT for the indicated time periods. As shown in Figure 2A, DHT incubation markedly elevated Akt phosphorylation levels in SMMC-7721 and L02, but not in HepG2 cells. Moreover, DHT-promoted Akt phosphorylation was blocked by PI3K inhibitor LY294002 in SMMC-7721 and L02 cells (Supplementary Fig. 2). It is notable that DHT initiated Hsp27 and GADD153 induction in SMMC-7721 and L02, but not HepG2 cells (Fig. 2B, C). These data suggest that SMMC-7721 and L02 are androgen-sensitive liver cells.

To investigate whether GADD153, Hsp27, and PI3K/Akt were involved in DHT-induced cell-cycle arrest, DHT-induced GADD153 and Hsp27 expression were suppressed by siRNA. Moreover, PI3K inhibitor LY294002 was used to block DHT-mediated PI3K/Akt activation. Both GADD153 and Hsp27 suppression inhibited DHT-induced cell cycle arrest in SMMC-7721 (Fig. 2D) and L02 (Fig. 2E) cells. However, PI3K inhibitor LY294002 had no demonstrable effect on the cell cycle progression in response to DHT treatment (data not shown). These data suggest that the induction of GADD153 and Hsp27 is involved in DHT-initiated cell cycle arrest.

DHT INDUCES PKR/eIF2α ACTIVATION

Since GADD153 can be induced by ER stress response, it is possible that DHT may elicit ER stress response. To test this possibility, the mRNA levels of typical ER stress response biomarkers (XBP1, GRP78, and GADD153) were determined upon DHT treatment. We found that DHT markedly elevated the mRNA levels of GADD153, but had no effect on the mRNA of GRP78 and spliced XBP1 induction (Fig. 3A). It is notable that DHT resulted in eIF2α phosphorylation on serine 51 in SMMC-7721 and L02 cells without GRP78 protein induction (Fig. 3B). In contrast, ER stress inducer Tg markedly elevated the expression of GRP78 and induced XBP1 mRNA splicing in SMMC-7721 and L02 cells (Fig. 3A,B). Thus, DHT activates the eIF2α/GADD153 pathway independent of ER stress response induction.

We next tested whether PKR is involved in DHT-induced eIF2α phosphorylation. SMMC-7721 and L02 cells were incubated with PKR inhibitor C16 1 h before the addition of DHT. The results showed

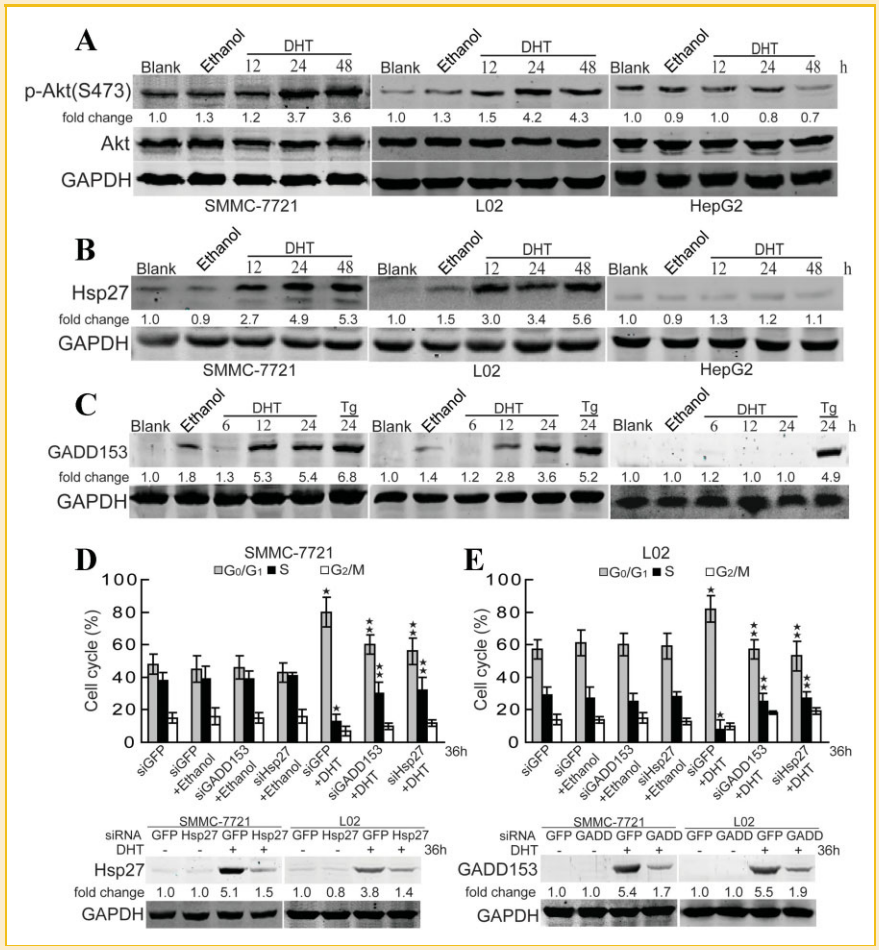

Fig. 2. GADD153 and Hsp27 are involved in DHT-induced cell cycle arrest. A–C: The effect of DHT on *p*-Akt, GADD153, and Hsp27 expression. SMMC-7721, L02, and HepG2 cells with DHT (100 nM) treatment for indicated time periods were subjected to western blot analysis for the expression of *p*-Akt (A), Hsp27 (B), and GADD153 (C). D, E: The suppression of GADD153 and Hsp27 inhibits DHT-induced cell cycle arrest. After transfected with GADD153 and Hsp27 siRNA for 12 h, SMMC-7721 (D) and L02 (E) cells were treated with DHT (100 nM) for another 36 h and then subjected to measurement of cell cycle distribution using flow cytometry. Data are presented as mean values ± SD of three measurements. Columns, mean of three individual experiments; bars, SE. *Significantly different from control value; **significantly different from *value.

that C16 inhibited DHT-induced eIF2α phosphorylation (Fig. 3C). To confirm the role of PKR in DHT-mediated eIF2α phosphorylation, the expression of PKR was suppressed by PKR-specific siRNA. Figure 3D shows that suppression of PKR substantially decreased eIF2α phosphorylation upon DHT treatment in SMMC-7721 and L02 cells. Taken together, these results suggest that DHT initiates PKR/eIF2α activation, which might be involved in GADD153 induction in androgen-sensitive liver cells.

PKR/eIF2α ACTIVATION IS INVOLVED IN DHT-MEDIATED GADD153 AND HSP27 INDUCTION

To investigate whether PKR is responsible for DHT-induced GADD153 expression, the expression of PKR was suppressed by PKR-specific siRNA in SMMC-7721 and L02 cells. Figure 4A shows that suppression of PKR significantly inhibited DHT-mediated GADD153 induction. Interestingly, DHT-induced Hsp27 expression also were substantially decreased in SMMC-7721 and L02 cells when suppression of PKR expression (Fig. 4A). Together, these data indicate that DHT might initiate GADD153 and Hsp27 induction through PKR/eIF2α activation.

To make sure the role of eIF2α phosphorylation in GADD153 and Hsp27 induction, small molecule drug salubrinal (Sal), which selectively blocks dephosphorylation of eIF2α but not other PP1 substrates [Boyce et al., 2005] was used in our study. We found that Sal treatment initiated GADD153 and Hsp27 induction in SMMC-7721 and L02 cells (Fig. 4B). These results suggest that the PKR/eIF2α signaling pathway is involved in DHT-induced GADD153 and Hsp27 expression.

PKR/eIF2α ACTIVATION IS INVOLVED IN DHT-INDUCED CELL CYCLE ARREST

As our data demonstrated that GADD153 and Hsp27 induction contribute to DHT-induced cell cycle arrest and PKR/eIF2α activation is involved in DHT-induced GADD153 and Hsp27 expression, we addressed whether PKR/eIF2α activation regulates cell cycle progression in liver cells. As depicted in Figure 5A,B, PKR knockdown inhibited DHT-induced cell cycle arrest in SMMC-7721

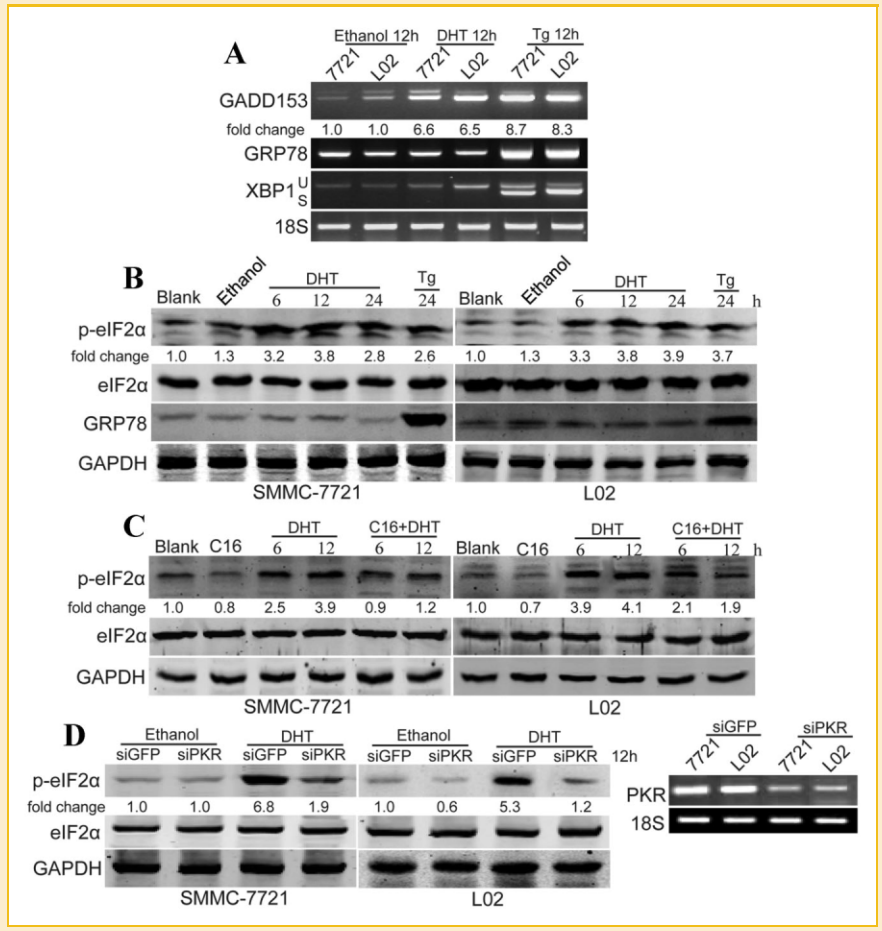

Fig. 3. DHT induces PKR/eIF2α activation. A: DHT initiates GADD153 expression without ER stress response activation. SMMC-7721 and L02 cells were treated with DHT (100 nM) for 12 h and then subjected to RT-PCR analysis. The shorter band (S) and longer band (U) indicated the spliced form (active form) and un-spliced form (inactive form) of XBP1 mRNA, respectively. Tg treatment was used as positive control. B: DHT induces eIF2α phosphorylation. DHT (100 nM)-treated SMMC-7721 and L02 cells were subjected to western blot analysis. Tg treatment was used as positive control. C, D: PKR responsible for DHT-induced eIF2α phosphorylation. After treated with DHT (100 nM) for indicated time periods with or without C16 (1 μM) (C) or PKR siRNA (D) pretreatment, SMMC-7721 and L02 cells were subjected to western blot analysis.

and L02 cells. Furthermore, Sal treatment induced an increase of the percentage of G_0/G_1 phase cells, with a concomitant decreases in the percentage of S phase cells in SMMC-7721 and L02 cells (Fig. 5A,B). Together, these data suggest that PKR/eIF2α activation mediates DHT-induced cell cycle arrest in androgen-sensitive liver cells.

GADD153 IS INVOLVED IN DHT-INDUCED APOPTOSIS

To investigate the effect of DHT on liver cells apoptosis, DHT-treated SMMC-7721, L02, and HepG2 cells were subjected to apoptosis analysis by western blot and Annexin V-FITC flow cytometry. As shown in Figure 6A, apoptosis were detected in DHT-treated SMMC-7721 and L02 cells. However, DHT treatment had no demonstrable effect on HepG2 cells apoptosis (data not shown).

Since previous findings indicated that GADD153 plays a critical role in inducing liver cells apoptosis under certain conditions [Benali-Furet et al., 2005], it is possible that DHT may initiates liver cells apoptosis through GADD153 induction. To test this possibility, SMMC-7721 and L02 cells were transiently transfected with GADD153 siRNA 12 h before DHT treatment. The results showed that GADD153 suppression decreased the sensitivity of SMMC-7721 and L02 cells to DHT-induced apoptosis (Fig. 6B), suggesting that DHT can initiate androgen-sensitive liver cells apoptosis through the PKR/eIF2α/GADD153 pathway.

FLUTAMIDE INHIBITS DHT-INDUCED CELL CYCLE ARREST AND APOPTOSIS

To confirm the effect of DHT in initiating cell cycle arrest and apoptosis via AR in SMMC-7721 and L02 cells, the AR antagonist Flu was used in our study. As shown in Figure 7A,B, Flu blocked DHT-induced cell cycle arrest and apoptosis in SMMC-7721 and L02 cells. The analysis of these data sets confirmed our suggestion that DHT initiates cell cycle arrest and apoptosis via AR in androgen-sensitive liver cells.

DISCUSSION

Mounting data indicate that AR-mediated signaling plays a pivotal role in the pathogenesis of various liver diseases, such as NAFLD and HCC [Lin et al., 2008; Ma et al., 2008; Vassilatou et al., 2010; Wu et al., 2010]. In this study, we demonstrated that DHT initiates cell

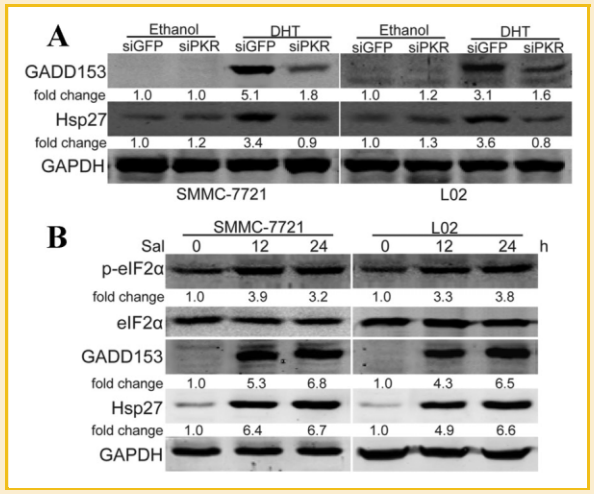

Fig. 4. PKR/eIF2α is involved in DHT-mediated GADD153 and Hsp27 induction. A: PKR is involved in DHT-mediated GADD153 and Hsp27 induction. After transfected with PKR siRNA for 24 h, SMMC-7721, and L02 cells were treated with DHT (100 nM) for another 24 h and then subjected to western blot analysis. B: Sal induces GADD153 and Hsp27 expression. After treated with Sal (25 μM) for indicated time periods, SMMC-7721 and L02 cells were subjected to western blot analysis.

cycle arrest and apoptosis, at least in part, via PKR/eIF2α activation in androgen-sensitive human liver cells.

DHT results in cell cycle arrest in androgen-sensitive liver cells, such as SMMC-7721 and L02. However, HepG2 cells, which also express high level of AR, are not sensitive to DHT. Further studies are needed to investigate if the insensitivity of AR in response to DHT in HepG2 cells was due to a mutation or some other reason. Since AR signaling can activate the PI3K/Akt pathway [Sun et al., 2003; Baron et al., 2004], which is implicated in the regulation of cell cycle progression [Kandel et al., 2002; Shtivelman et al., 2002], it raises the possibility that PI3K/Akt may participate in regulating DHT-induced cell cycle arrest. However, our results demonstrated that DHT-mediated PI3K/Akt activation is not involved in cell cycle regulation in DHT-treated liver cells (data not shown).

Importantly, DHT induced the expression of GADD153 and Hsp27, and which contributed to DHT-mediated cell cycle arrest in androgen-sensitive liver cells. Since GADD153 is the target of eIF2α phosphorylation, and the eIF2α/GADD153 pathway is a branch of ER stress response, we asked whether DHT induces ER stress response. Based on our data that DHT initiates eIF2α phosphorylation on S51 and GADD153 induction without other potential biomarkers of ER stress activation (such as XBP1 activation and GRP78 induction), we propose that DHT-mediated eIF2α/GADD153 activation independent of ER stress.

Considering that PKR inhibits protein synthesis by phosphorylating eIF2α on Ser51 [Taylor et al., 2005], we investigated whether PKR account for DHT-initiated eIF2α/GADD153 activation. Based on the data that both PKR inhibition and knockdown inhibit DHT initiated eIF2α/GADD153 activation, we propose that PKR responsible for the eIF2α/GADD153 pathway activation in response to DHT treatment. Furthermore, suppressing the expression of PKR also inhibits DHT-induced Hsp27 induction, indicating that DHT induces Hsp27 expression, at least in part, through PKR/eIF2α activation. As PKR, GADD153, or Hsp27 suppression blocks DHT-induced cell cycle arrest, it is reasonable that PKR/eIF2α activation contributes to DHT-mediated cell cycle arrest. These results, when taken together, indicate that the activation of PKR/eIF2α is involved in DHT mediated cell cycle arrest, at least in part, through GADD153 and Hsp27 induction. Further studies are needed to investigate the detailed mechanisms that link DHT/AR signal and PKR/eIF2α activation.

In addition to its role in cell cycle arrest induction, DHT also is implicated in apoptosis initiation in androgen-sensitive liver cells. As GADD153 is a key inducer of cell apoptosis in certain conditions, it is possible that DHT can induce androgen-sensitive liver cells apoptosis through PKR/eIF2α/GADD153 signaling cascades. This hypothesis was supported by the data, which demonstrated that GADD153 suppression inhibits SMMC-7721 and L02 cells apoptosis in response to DHT. DHT induced survival signal PI3K/Akt activation and Hsp27 accumulation, but these are overwhelmed by the dominant role of the PKR/eIF2α/GADD153 cascades in apoptosis induction in DHT-treated SMMC-7721 and L02 cells.

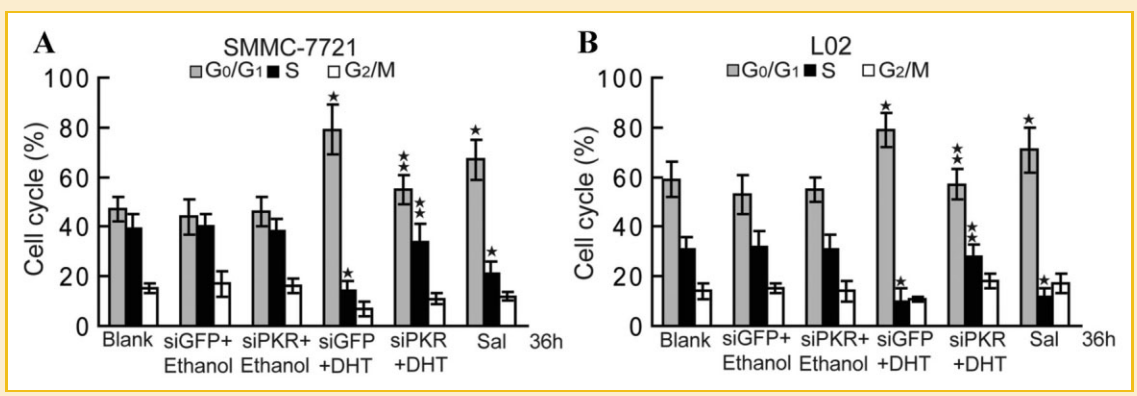

Fig. 5. PKR/eIF2α is involved in DHT-induced cell cycle arrest. After transfected with PKR siRNA for 24 h or treated with Sal, SMMC-7721 (A) and L02 (B) cells were treated with or without DHT (100 nM) for another 36 h and then subjected to measurement of cell cycle distribution using flow cytometry. Data are presented as mean values ± SD of three measurements. Columns, mean of three individual experiments; bars, SE. *Significantly different from control value; **significantly different from *value.

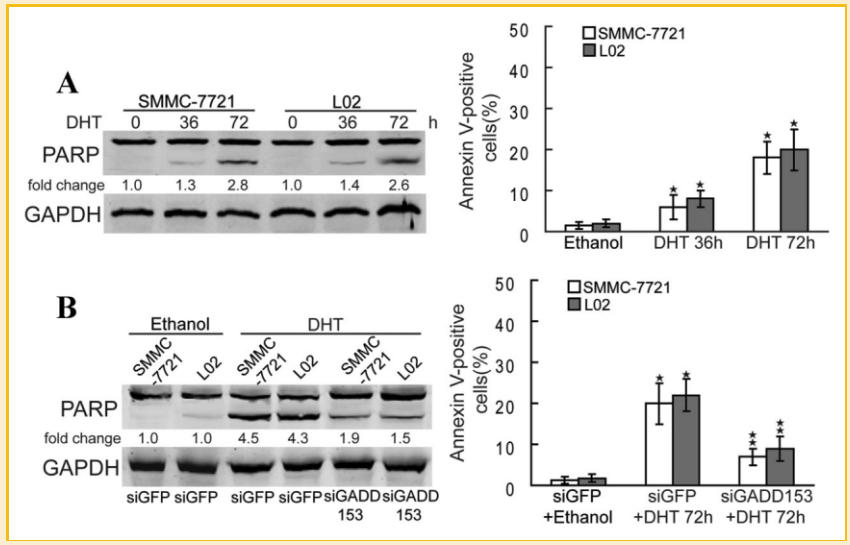

Fig. 6. GADD153 is involved in DHT-initiated apoptosis. A: DHT initiates apoptosis in liver cell lines. After treated with DHT (100 nM) for indicated time periods, SMMC-7721 and L02 cells were subjected to apoptosis analysis. B: GADD153 suppression inhibits DHT-induced apoptosis. After transfected with GADD153 siRNA for 12 h, SMMC-7721 and L02 cells were treated with DHT (100 nM) for another 72 h and then subjected to apoptosis analysis. Apoptosis was evaluated by PARP cleavage and reconfirmed by Annexin V-FITC flow cytometry. Columns, mean of three individual experiments; bars, S.E. *Significantly different from control value; **significantly different from *value.

Liver cell death is emerging as a prominent feature in many liver diseases, such as acute hepatic failure (AHF), NAFLD, and HCC [Zhao and Zimmermann, 1997; Nanji, 1998; Schuchmann and Galle, 2001; Jaeschke et al., 2004; Canbay et al., 2005; Guicciardi and Gores, 2010]. Considering that ER stress is implicated in liver disease and PI3K/Akt, Hsp27, and GADD153 have been well documented in regulating ER stress-induced apoptosis [Hu et al., 2004; Ito et al., 2005; Szegezdi et al., 2006; Malhi and Kaufman, 2011], it seems that AR signaling might regulate ER stress-mediated apoptosis in androgen-sensitive liver cells. This speculation is supported by our

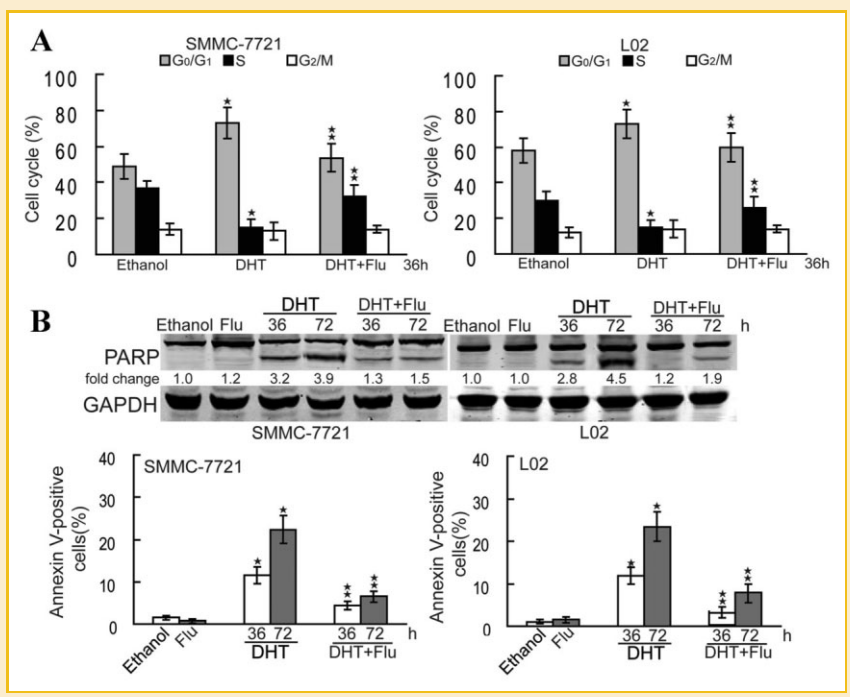

Fig. 7. Flu blocks DHT-initiated cell cycle arrest and apoptosis. SMMC-7721 and L02 cells were treated with DHT (100 nM) for indicated time periods with or without Flu (10 mM) pre-incubation for 1 h and then subjected to cell cycle distribution (A) and apoptosis (B) analysis. Columns, mean of three individual experiments; bars, S.E. *Significantly different from control value; **significantly different from *value.

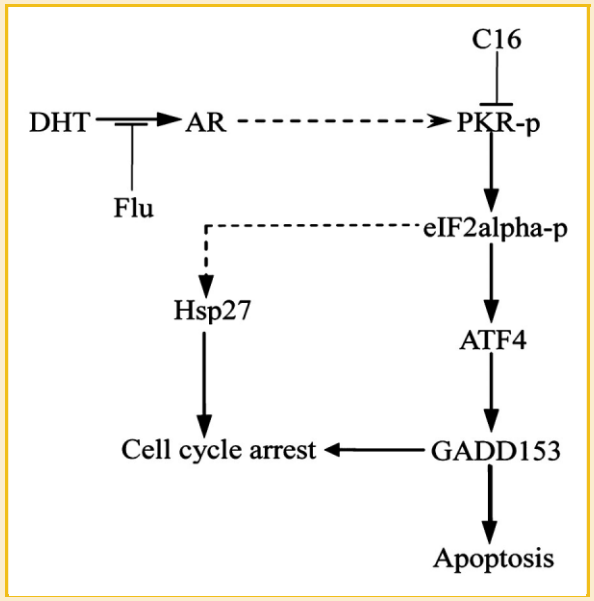

Fig. 8. Schematic summarizing the mechanisms by which DHT/AR signal initiates cell cycle arrest and apoptosis.

data, which demonstrated that DHT sensitizes SMMC-7721 and L02 cells to ER stress-mediated apoptosis (Supplementary Fig. 3). Further studies are required to reveal the detailed role of DHT-induced apoptosis and DHT-enhanced apoptosis upon ER stress in the development of certain liver diseases.

In brief, the present work reveals that DHT/AR signal cascades initiates liver cells apoptosis and cell cycle arrest, at least in part, via PKR/eIF2α activation (Fig. 8). Further studies on the function of androgen/AR on liver cells will contribute to the understanding of molecular mechanisms of liver diseases and the development of new therapeutic strategies against certain liver diseases.

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