EMBO reports

EMBO open

scientific report

Transcriptional activation of DNA-dependent protein kinase catalytic subunit gene expression by oestrogen receptor-α

Senad Medunjanin[1], Sönke Weinert[1], David Poitz[1], Alexander Schmeisser[2], Ruth H. Strasser[1] & Ruediger C. Braun-Dullaeus[2+]

[1]Division of Cardiology, Department of Internal Medicine, Dresden University of Technology, Dresden, Germany, and [2]Division of Cardiology, Department of Internal Medicine, Angiology and Pneumology, Magdeburg University, Magdeburg, Germany

The cellular response to DNA double-strand break (DSB) occurs through an integrated sensing and signalling network that maintains genomic stability. Oestrogen (E2), among its many functions, is known to have a positive effect on global genomic DNA repair; however, the mechanism by which it functions is unclear. A central enzyme involved in DNA DSB repair in mammalian cells is the DNA-dependent protein kinase (DNA-PK). Here, we show that E2 enhances DNA-PK catalytic subunit (DNA-PKcs) promoter activity with subsequent transcriptional and translational upregulation of DNA-PKcs in a breast cancer cell line. We identify two potential E2 receptor-α (ERα)-binding sites in a region upstream from the DNA-PKcs initiation site. By using small interfering RNA and the specific E2 receptor antagonist ICI 182,780, we demonstrate that ERα knockdown reduces E2-induced upregulation of DNA-PKcs expression and activity in breast carcinoma cells. E2-induced DNA-PK transactivation results in an increased ability of the cells to repair DNA DSB. This previously unknown mechanism of DNA-PK regulation sheds new light on tumour biology and reveals new possibilities for the prevention and therapy of E2-sensitive proliferative diseases.

Keywords: oestrogen; oestrogen receptor; DNA double-strand break; DNA-dependent protein kinase; ionizing radiation

EMBO *reports* (2010) 11, 208–213. doi:10.1038/embor.2009.279

[1]Division of Cardiology, Department of Internal Medicine, Dresden University of Technology, Fetscherstrasse 74, 01307 Dresden, Germany

[2]Division of Cardiology, Department of Internal Medicine, Angiology and Pneumology, Magdeburg University, Leipziger Strasse 44, 39120 Magdeburg, Germany

[+]Corresponding author. Tel: +49 391 6713203; Fax: +49 391 6713202; E-mail: r.braun-dullaeus@med.ovgu.de

Received 12 June 2009; revised 1 December 2009; accepted 4 December 2009; published online 29 January 2010

INTRODUCTION

In addition to its widespread role in human physiology, oestrogen (E2) is implicated in the development and progression of proliferative disorders such as breast cancer (Deroo & Korach, 2006). E2 exerts its effects through the E2 receptors (ERs) ERα and ERβ. ERα is a member of the class I nuclear hormone receptor superfamily. On ligand binding, the receptor forms homodimers that bind E2-responsive elements (EREs) located in the regulatory region of target genes (Martinez & Wahli, 1989). Although the effects of E2 on the proliferation of human breast cancer cells have been known for many years, only recently have gene expression profiling studies suggested a role of the hormone in DNA repair (Gadal *et al*, 2005).

DNA-dependent protein kinase (DNA-PK) is a serine/threonine protein kinase comprising a catalytic subunit (DNA-PKcs) and Ku subunits, which act as regulatory elements (Collis *et al*, 2005). DNA-PK is the main component of the non-homologous end-joining pathway of DNA double-strand break (DSB) repair in mammalian cells (Lees-Miller, 1996). It has been proposed that DNA-PK is a molecular sensor of DNA damage, which enhances DSB repair through the phosphorylation of many downstream targets (Anderson & Lees-Miller, 1992; Kysela *et al*, 2005; Hah *et al*, 2007). The crucial role of DNA-PK is to repair DSBs that either arise endogenously during normal cellular processes or are exogenously caused by genotoxic agents such as ionizing radiation (IR). Unrepaired DSBs are known to trigger cell-cycle checkpoint arrest and cell death (Norbury & Hickson, 2001). Recently, we demonstrated that glycogen synthase kinase 3 stabilizes ERα and modulates its transcriptional activity (Medunjanin *et al*, 2005). In addition, co-immunoprecipitation studies revealed the involvement of a 70 kD protein that was subsequently identified as Ku70, a component of the DNA-PK holoenzyme. When studying the role of DNA-PK in ERα activity, increased

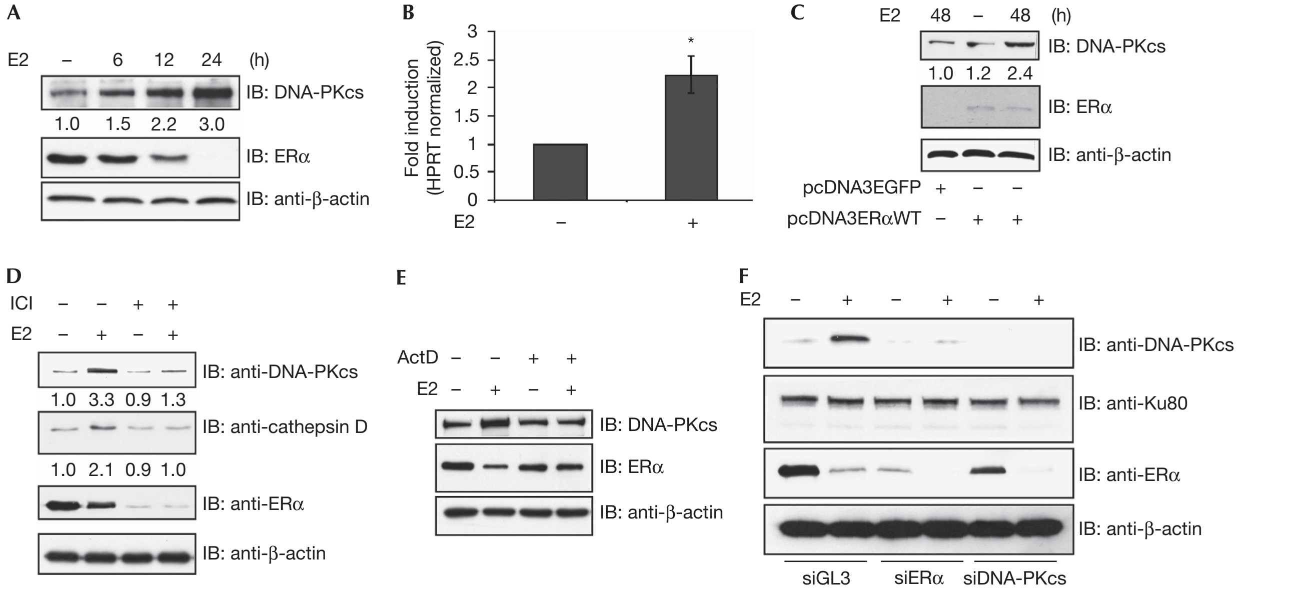

Fig 1 | E2-dependent induction of DNA-PKcs. (**A**) Protein extracts from MELN cells treated for different time periods with 100 nM of E2 were analysed for the expression of DNA-PKcs by immunoblotting. β-Actin was used as a loading control. Numbers indicate the ratio of DNA-PKcs protein in untreated cells compared with E2-treated cells. (**B**) MELN cells were treated with 100 nM of E2 and analysed for the expression of DNA-PKcs by quantitative RT–PCR. $*P<0.05$. (**C**) The plasmids were transiently transfected into the COS-7 cells. After 24 h, cells were stimulated with 10 nM of E2 for 48 h, then cells were collected and proteins were detected by immunoblotting. (**D**) MCF-7 cells were grown in phenol red-free medium supplemented with 10% charcoal–dextran-stripped FBS for 3 days. After pretreatment with 1 μM of the ERα antagonist ICI for 24 h, cells were treated with 100 nM of E2 for 24 h and proteins were immunoblotted with the indicated antibodies. Numbers indicate the ratio of DNA-PKcs and cathepsin D protein in untreated cells compared with E2-treated cells. (**E**) MCF-7 cells were grown in phenol red-free medium supplemented with 10% charcoal–dextran-stripped FBS for 3 days. After pretreatment with 0.1 μM of ActD for 24 h, cells were treated with 100 nM of E2 for 24 h. (**F**) MCF-7 cells were transfected either with GL3 control siRNA (siGL3) or with siRNA targeting DNA-PKcs and ERα. The cells were treated—or not—with 100 nM of E2 for 48 h, followed by immunoblotting with specific antibodies as indicated. ActD, actinomycin D; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; E2, oestrogen; ERα, oestrogen receptor-α; FBS, fetal bovine serum; HPRT1, hypoxanthine-guanine phosphoribosyltransferase 1; IB, immunoblotting; ICI, ICI 182,780; RT–PCR, reverse transcriptase PCR; siRNA, small interfering RNA.

DNA-PKcs expression levels were observed in cells treated with E2. We thus tested the hypothesis that DNA-PKcs is a direct target of ERα.

RESULTS

E2-dependent induction of DNA-PKcs

To test our hypothesis, cells from the breast cancer cell line MELN were treated with E2 in a time-dependent manner, which resulted in a marked upregulation of DNA-PKcs and an expected downregulation of ERα within 6 h (Fig 1A). Quantitative real-time reverse transcriptase PCR revealed that E2 induced a significant—about twofold—induction of DNA-PKcs messenger RNA expression that peaked at 1 h (Fig 1B). This was confirmed by transfection of ERα–Flag fusion protein into ERα-negative COS-7 cells yielding upregulation of DNA-PKcs after E2 treatment only in ERα-positive cells (Fig 1C). E2-dependent induction of DNA-PKcs was reduced markedly with either the specific ER antagonist ICI 182,780 (Fig 1D) or the transcriptional repressor actinomycin D (ActD; Fig 1E). Furthermore, we used small interfering RNA (siRNA) to knockdown ERα levels. In the presence of the specific siRNA, DNA-PKcs protein levels were reduced (Fig 1F).

Oestrogen transactivates the DNA-PKcs promoter

Subsequently, the promoter region of DNA-PKcs was analysed for EREs. The 13 bp palindromic ERE with the consensus sequence 5′-GGTCANNNTGACC-3′ is well known (Klein-Hitpass *et al*, 1988). However, it has been shown that 'imperfect' palindromic EREs might also act synergistically to permit translational activity (Martinez & Wahli, 1989). Analysis of the DNA-PKcs promoter demonstrated ERE-binding elements located in close proximity to each other: an 'imperfect' palindromic ERE at position −184 and a half palindromic ERE at position −274 (Fig 2A). First, we cloned a previously described fragment of the DNA-PKcs promoter (from −3 to −606; Connelly *et al*, 1998) into the pGL3-Basic luciferase reporter plasmid and tested for promoter activity 72 h after transient transfection of human HeLa and MCF-7 cells. As HeLa cells lack a detectable endogenous ER, ERα was co-transfected with the DNA-PKcs reporter gene. Co-transfection induced a threefold increase in luciferase activity that increased further on E2 treatment (Fig 2B). Transfection of the DNA-PKcs reporter into the ERα-positive cell line MCF-7 was also accompanied by a strong luciferase signal that was sensitive to E2 stimulation. According to the above promoter analysis, the promoter region of DNA-PKcs was subsequently restricted to position −220 upstream from the

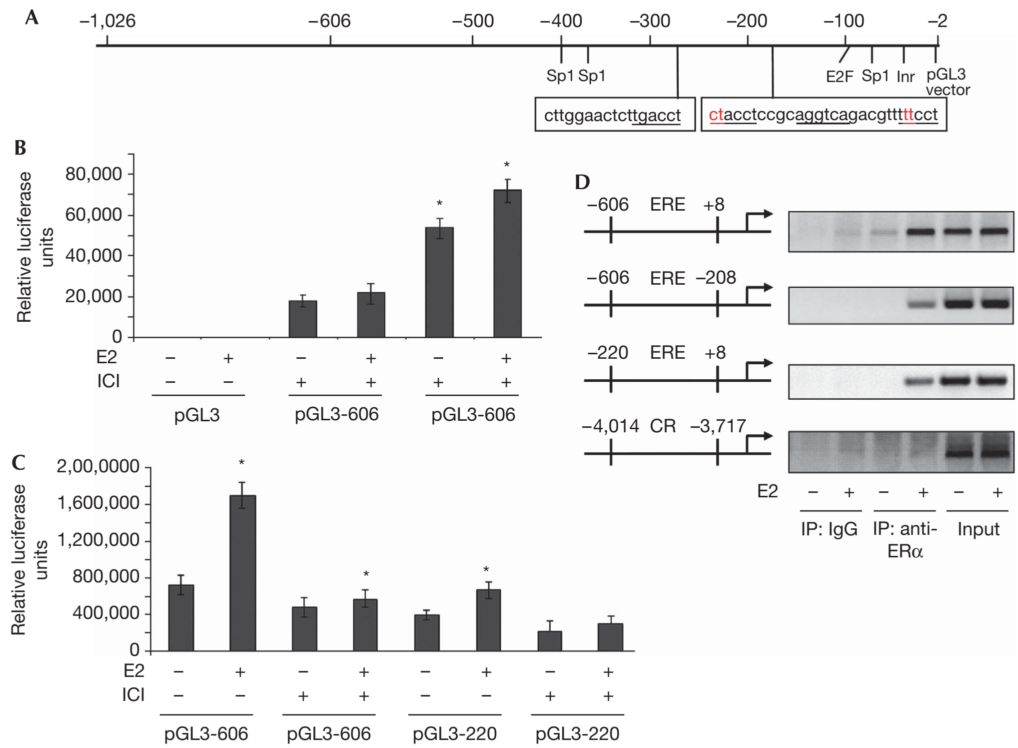

Fig 2 | Analysis of the human DNA-PKcs promoter in HeLa and MCF-7 Cells. (**A**) DNA-PKcs promoter region showing putative binding sites for ERα, Sp1 and a potential initiator consensus element (Inr). Differences to the core 13 bp consensus ERE are labelled in red. (**B**) HeLa cells were transfected with the DNA-PKcs promoter luciferase reporter plasmid and ERα expression plasmid and treated with 100 nM of E2 for 24 h. The luciferase activities were normalized to the internal transfection control and values obtained from cells receiving vehicle (−) were set as 1. Empty pGL3-promoter vector was used as a negative control. Data represent the mean of three independent experiments performed in triplicate. *$P<0.05$. (**C**) MCF-7 cells were transfected with DNA-PKcs promoter luciferase reporter plasmids and treated with 100 nM of E2 for 24 h. The luciferase activities were normalized to the internal transfection control, and values obtained from cells receiving vehicle (−) were set as 1. Data represent the mean of three independent experiments performed in triplicate. *$P<0.05$. (**D**) Cells were grown in phenol red-free medium supplemented with 10% charcoal–dextran-stripped FBS for 3 days. After treatment with 100 nM of E2 for 1 h, cells were crosslinked with 1% formaldehyde and monitored by ChIP assays. Soluble chromatin from control and E2-treated MCF-7 cells was immunoprecipitated with anti-ERα or a control IgG. The final DNA extractions were amplified by PCR using pairs of primers that cover the indicated EREs or CR of the DNA-PKcs promoters. ChIP, chromatin immunoprecipitation; CR, control region; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; E2, oestrogen; ERα, oestrogen receptor-α; ERE, oestrogen-responsive element; FBS, fetal bovine serum; ICI, ICI 182,780; IgG, immunoglobulin G; IP, immunoprecipitation.

DNA-PKcs initiation site, cloned into the luciferase reporter plasmid and examined for promoter activity 72 h after E2 stimulation, confirming the relevance of the hypothesized EREs in DNA-PKcs transactivation. E2-induced increase in luciferase activity was reduced when the cells had been preincubated with the anti-E2 ICI 182,780 before stimulation (Fig 2C).

Binding of ERα to the DNA-PKcs promoter was further confirmed by chromatin immunoprecipitation assays with ERα antibody. In MELN cells, occupancy of the DNA-PKcs promoter by ERα was not detected in the absence of E2, but increased markedly on E2 stimulation (Fig 2D, upper panel). In subsequent experiments, we amplified a portion of human DNA-PKcs promoter bearing a putative half or imperfect ERE. The ERα binding to these sites was reduced markedly after E2 treatment (Fig 2D). Immunoprecipitations performed with either immunoglobulin G (IgG) or a control region (from −4,014 to −3,717) of the DNA-PKcs promoter upstream from the ERE were used as controls. Together, these findings indicate that: (i) ERα directly binds to the DNA-PKcs promoter and (ii) E2 stimulation is associated with increased occupancy of EREs by ERα.

Oestrogen-induced double-strand break repair

Increased expression of DNA-PKcs alone might provide important protection from genotoxic stress, even in cells already containing functional DNA-PK (Shen *et al*, 1998). To determine the functional significance of our finding, we examined the effect of E2 on DNA DSB repair in human MELN cells by studying the kinetics of γ-H2AX foci formation, which result from DSBs during irradiation (Sedelnikova *et al*, 2002; Rothkamm & Lobrich, 2003). Time course experiments revealed that the number of γ-H2AX foci reached its maximum 1 h after irradiation of MELN cells. After irradiation, it fell almost to control level within 6 h (Fig 3A). Foci formation was reduced significantly when cells were exposed to E2 before irradiation (Fig 3A). As foci formation takes a finite

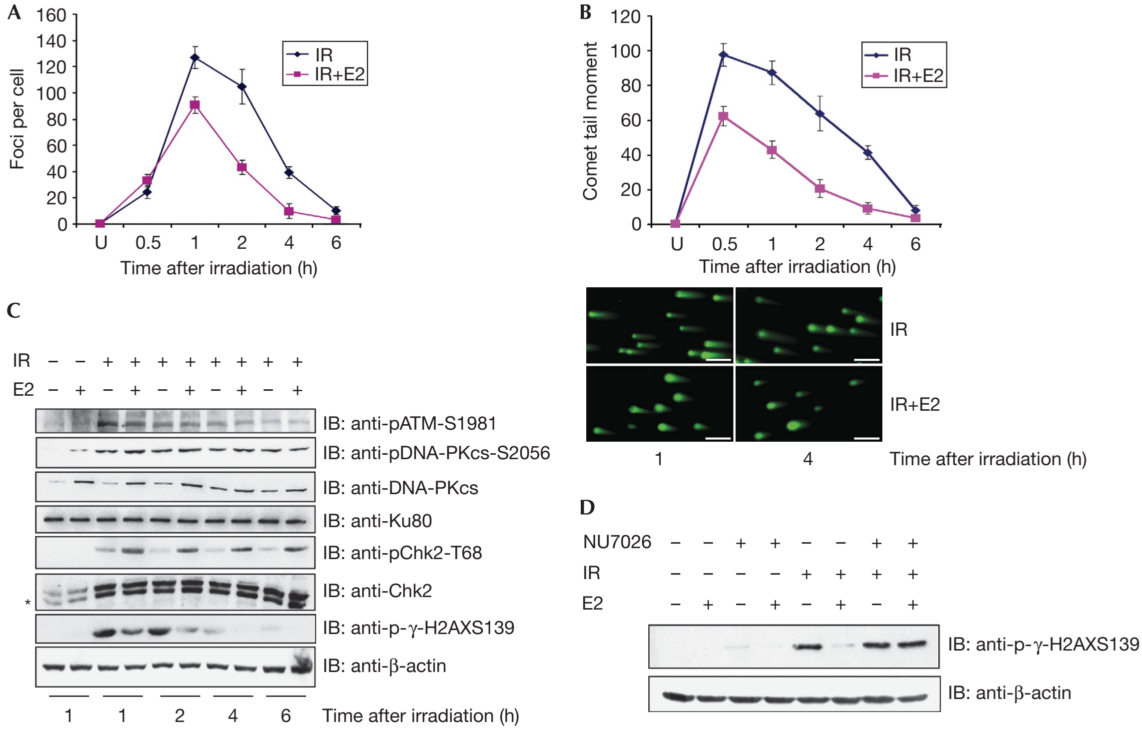

Fig 3 | E2-induced DSB repair. (**A**) MELN cells were grown in phenol red-free medium supplemented with 10% charcoal–dextran-stripped FBS for 3 days. After pretreatment with 100 nM of E2 for 20 h, cells were left untreated or treated with IR (10 Gy). Cells were fixed for immunofluorescent staining with the γ-H2AX antibody (Ser 139) as a marker for DSBs. Foci were counted in at least 50 cells per condition. Error bars represent the s.d. values from at least three independent experiments. Differences were statistically significant at the $P<0.05$ level. (**B**) MELN cells were grown as above. After ± pretreatment with 100 nM of E2 for 20 h, cells were exposed to IR (20 Gy) and incubated for different times. The distribution of tail moments among a population of 100 cells for each time point was plotted. Error bars represent the s.d. values from at least three independent experiments. Representative images are shown additionally. Scale bars, 100 μm. (**C**) MELN cells were treated as in (**A**) followed by immunoblotting with specific antibodies as indicated. The asterisk represents an unspecific band recognized by the Chk2 antibody. (**D**) MELN cells were grown in phenol red-free medium supplemented with 10% charcoal–dextran-stripped FBS for 3 days. After pretreatment with 5 μM of NU7026 and 100 nM of E2 for 20 h, cells were treated with IR (10 Gy) and incubated for 2 h. Cell lysates were assayed for expression of proteins with the indicated antibodies. β-Actin was used as a loading control. ATM, ataxia teleangiectasia mutated; Chk2, checkpoint kinase 2; DSB, double-strand break; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; E2, oestrogen; ERα, oestrogen receptor-α; FBS, fetal bovine serum; γ-H2AX, phosphorylated histone H2AX; IB, immunoblotting; IR, ionizing radiation; U, untreated.

amount of time, this assay was not able to assess the initial number of DSBs. Thus, DSB induction and repair after IR was additionally visualized and quantified by using the neutral COMET assay. Pretreatment of MELN cells with E2 significantly increased the repair of damaged DNA—that is, decreased the comet tail moment—after irradiation (Fig 3B).

Western blot analyses visualized reduced phosphorylation of γ-H2AX in E2-treated cells compared with untreated cells and confirmed the results of the immunofluorescence microscopy (Fig 3C). A previous study (Li & Stern, 2005) indicated that phosphorylation of DNA repair protein checkpoint kinase 2 (Chk2) at Thr 68 depends on the activation of DNA-PK after irradiation. Therefore, phosphorylation of Chk2 at Thr 68 was used to show that DNA-PK had indeed been activated. By contrast, phosphorylation of ataxia teleangiectasia mutated (ATM), another central kinase of DSB repair, was not sensitive to E2 treatment (Fig 3C). When DNA-PK had been inhibited, E2 lost its preventive effect on γ-H2AX foci formation after IR (Fig 3D). Involvement of ERα in DSB repair was further supported by using the specific ER antagonist ICI 182,780. E2-dependent induction of DNA-PKcs was reduced markedly when MELN cells were preincubated with the anti-E2 ICI 182,780 before irradiation. Consequently, E2-induced increased phosphorylation of Chk2 at Thr 68 was prevented markedly in ICI-treated cells (Fig 4A).

Previous studies demonstrated that ATM- and Rad3-related (ATR), another PI3-related kinase, also regulate phosphorylation of Chk2 at Thr 68 (Matsuoka *et al*, 2000; Wang *et al*, 2006). To rule out ATM and ATR as activators of Chk2, we used the specific ATM/ATR kinase inhibitor CGK733. No reduction in the phosphorylation of Chk2 at Thr 68 after E2 treatment was observed when the ATM/ATR inhibitor was used (Fig 4B).

DISCUSSION

In recent years, mammalian DNA-PKcs has been shown to be a crucial component of the signalling mechanisms of both DSB repair and V(D)J recombination (Shen *et al*, 1998), which was

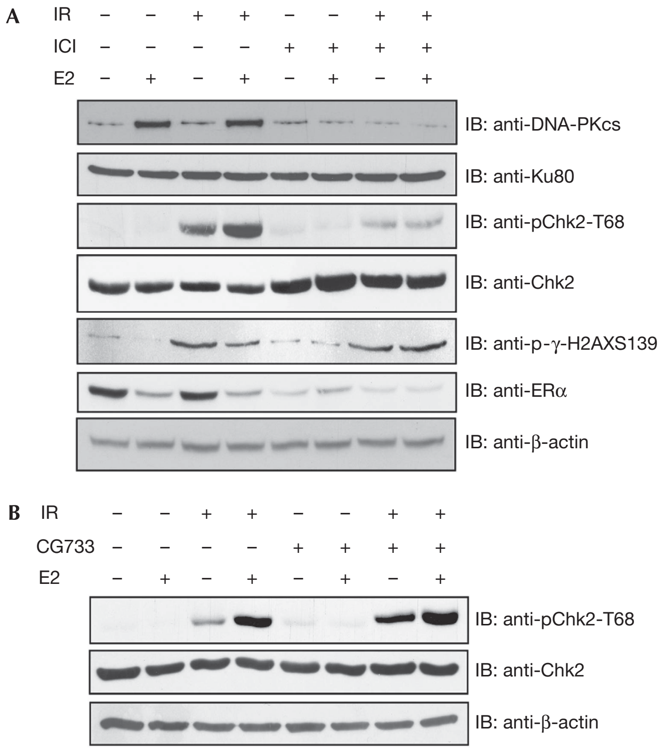

Fig 4 | ER antagonist ICI 182,780 reduced E2-dependent induction of DNA-PKcs. (**A**) MELN cells were grown in phenol red-free medium supplemented with 10% charcoal–dextran-stripped FBS for 3 days. After pretreatment with 1 μM of ICI 182,780 and 100 nM of E2 for 20 h, cells were treated with IR (10 Gy) and incubated for 2 h. Cell lysates were assayed for expression of proteins with the indicated antibodies. (**B**) MELN cells were grown as above. After ± pretreatment with 10 μM of CGK733 and 100 nM of E2 for 20 h, cells were exposed to IR (10 Gy) and incubated for 2 h. Cell lysates were assayed for expression of proteins with the indicated antibodies. Chk2, checkpoint kinase 2; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; E2, oestrogen; ERα, oestrogen receptor-α; FBS, fetal bovine serum; IB, immunoblotting; IR, ionizing radiation.

further supported by the finding that a point mutation in the carboxy terminus of DNA-PKcs leads to increased radiosensitivity of Chinese hamster ovary cells (Errami *et al*, 2000; Woods *et al*, 2002). Furthermore, severe combined immunodeficient (SCID) mice, which lack DNA-PKcs, exhibit increased susceptibility to IR and impaired V(D)J recombination (Smith & Jackson, 1999). This is the first report to show that DNA-PK can be induced directly by E2 through increased binding of the transcription factor ERα to the DNA-PKcs promoter. Furthermore, we characterized functional EREs in the DNA-PKcs promoter. Concerning this finding, it is interesting that the distance between the ERα half-sites is approximately within one nucleosome (∼200 bp), raising the possibility that they are in topographical proximity in the chromatin. Furthermore, the resulting DNA-PKcs promoter activity might be regulated tightly by a dynamic interplay between these two ERE sites.

A positive effect of E2 on global genomic repair of DNA and survival has been shown before (Boulay & Perdiz, 2005). Now, our data identify DNA-PK as the mediator of E2-induced DSB repair. Elevated levels of DNA-PK warrant a quick and strong response to DNA damage, resulting in reduced cellular levels of DSBs. An involvement of ATM, another important DNA repair enzyme, could be excluded in this context.

Clinically, the ERα has a central role in controlling cell growth in mammary tissue, and its expression level in breast cancer is associated with longer survival and a better response to therapeutic measures (Ruiz *et al*, 2006). However, through DNA-PK activation, ERα positivity might confer a selective advantage to tumour cells when exposed to radiotherapy or chemotherapeutic agents. Consequently, anti-E2s could sensitize cells for these therapies. Indeed, breast cancer cell lines incubated with tamoxifen were shown to be more sensitive to irradiation (Wazer *et al*, 1989), and the combination of radiotherapy and anti-hormonal therapy has been shown to prevent tumour recurrence (Fisher *et al*, 2002). Whether anti-hormonal pretreatment before radiotherapy improves treatment results in patients with breast cancer has, however, not yet been established. Nonetheless, our finding of an interaction between two central components of DNA replication and repair sheds further light on tumour biology and reveals new possibilities for the prevention and therapy of E2-sensitive proliferative diseases.

METHODS

Antibodies and reagents. The following antibodies, kit and inhibitors were used: anti-cathepsin D (4G2) and anti-α-actin (Abcam, Cambridge, UK); anti-ERα (MAB463; Chemicon, Bad Nauheim, Germany); anti-ERα (HC-20), anti-Ku80 (SC-1484) and anti-DNA-PKcs (sc-5282; Santa Cruz Biotechnology, Heidelberg, Germany); anti-Chk2 (#2662), anti-Chk2 (#3440) and DNA Damage Sampler Kit (#9947; New England BioLabs, Frankfurt am Main, Germany); alexa-green conjugated anti-mouse IgG (Molecular Probes, Eugene, OR, USA); ICI 182,780, ATM/ATR Kinase inhibitor (CGK 733), ActD and DNA-PK inhibitor (NU7026; Merck, Darmstadt, Germany).

Gene silencing with siRNAs. Transfection of MELN cells with siRNA has been described before (Grisouard *et al*, 2007). The siRNA oligonucleotides with 3′-TT overhangs were purchased from MWG-BIOTECH AG (Ebersberg, Germany). The following siRNA sequences were used: (siERα) 5′-AGGCUCAUUCCAGC CACAGTTdTdT-3′; and (siDNA-PKcs) 5′-CUUUAUGGUGGC CAUGGAGdTdT-3′. The concentration of siRNAs was 20 nM during transfection. For control, we used GL3-targeted siRNA.

Comet assays. The neutral COMET assay (single-cell gel electrophoresis assay) was performed according to the manufacturer's instructions (Trevigen, Gaithersburg, MD, USA). Briefly, after irradiation, cells were collected and mixed with low-melting agarose. After lysis, electrophoresis was performed at 1 V/cm and 15 mA for 40 min. Slides were stained with SYBR Green dye (Invitrogen, Karlsruhe, Germany) for 10 min. A total of 100 randomly selected cells per sample were captured under a Zeiss fluorescent microscope and digital fluorescent images were obtained using the AxioVision software (Carl Zeiss Imaging, Jena, Germany). The relative length and intensity of SYBR green-stained DNA tails to heads were proportional to the amount of DNA damage present in the individual nucleus and was measured by Olive tail moment with TriTek Comet Score software (TriTek, Sumerduck, VA, USA).

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation assays were performed as described previously (Wei *et al*, 2006). Briefly, lysates were sonicated with Branson Sonifer Cell Disruptor B15 (Branson, Danbury, CT, USA) to shear DNA to an average size of 600–1,000 bp. For chromatin immunoprecipitation, anti-ERα (MAB463) and IgG (Pierce, Bonn, Germany) were used. Purified DNA was amplified across the DNA-PKcs promoter region.

Luciferase assay. The MELN cells were washed with phosphate-buffered saline (Mg^{2+} and Ca^{2+} free) and lysed in 150 μl per well luciferase cell culture lysis reagent (Promega, Mannheim, Germany). Luciferase assays were performed by using the firefly luciferase assay system from Promega according to the manufacturer's instructions and quantified with a luminometer (LB9506, Berthold, Bad Wildbad, Germany).

Plasmids. The plasmids for ERα have been described previously (Medunjanin *et al*, 2005). Constructs of the DNA-PKcs promoter (GenBank accession number U63630) were produced by PCR amplification using genomic DNA from MELN cells. The resulting fragments, spanning the promoter region from positions −1,026, −606, −220 to position −2 relative to the transcriptional start site, were cloned into the *Nhe*I and *Xho*I cloning sites of the pGL3-Basic vector upstream from the firefly luciferase gene (Promega).

Statistical analysis. Data are presented as mean ± s.e.m. Statistical analysis was performed by analysis of variance. Post-test multiple comparisons were performed by using the Bonferroni method. All experiments were independently repeated at least three times.

Additional experimental procedures are described in the supplementary information online.

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

ACKNOWLEDGEMENTS
We thank C. Zufelde for valuable technical assistance. This study was supported by grants from Deutsche Forschungsgemeinschaft (DFG BR 1603/4 and SFB655/A11) and DFG Research Center for Regenerative Therapies.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

REFERENCES
Anderson CW, Lees-Miller SP (1992) The nuclear serine/threonine protein kinase DNA-PK. *Crit Rev Eukaryot Gene Expr* **2:** 283–314
Boulay F, Perdiz D (2005) 17β-estradiol modulates UVB-induced cellular responses in estrogen receptors positive human breast cancer cells. *J Photochem Photobiol B* **81:** 143–153
Collis SJ, DeWeese TL, Jeggo PA, Parker AR (2005) The life and death of DNA-PK. *Oncogene* **24:** 949–961
Connelly MA, Zhang H, Kieleczawa J, Anderson CW (1998) The promoters for human DNA-PKcs (PRKDC) and MCM4: divergently transcribed genes located at chromosome 8 band q11. *Genomics* **47:** 71–83
Deroo BJ, Korach KS (2006) Estrogen receptors and human disease. *J Clin Invest* **116:** 561–570
Errami A, Overkamp WJ, He DM, Friedl AA, Gell DA, Eckardt-Schupp F, Jackson SP, Hendrickson EA, Lohman PH, Zdzienicka MZ (2000) A new X-ray sensitive CHO cell mutant of ionizing radiation group 7,XR-C2, that is defective in DSB repair but has only a mild defect in V(D)J recombination. *Mutat Res* **461:** 59–69
Fisher B *et al* (2002) Tamoxifen, radiation therapy, or both for prevention of ipsilateral breast tumor recurrence after lumpectomy in women with invasive breast cancers of one centimeter or less. *J Clin Oncol* **20:** 4141–4149
Gadal F *et al* (2005) Integrative analysis of gene expression patterns predicts specific modulations of defined cell functions by estrogen and tamoxifen in MCF7 breast cancer cells. *J Mol Endocrinol* **34:** 61–75
Grisouard J, Medunjanin S, Hermani A, Shukla A, Mayer D (2007) Glycogen synthase kinase-3 protects estrogen receptor alpha from proteasomal degradation and is required for full transcriptional activity of the receptor. *Mol Endocrinol* **21:** 2427–2439
Hah YS, Lee JH, Kim DR (2007) DNA-dependent protein kinase mediates V(D)J recombination via RAG2 phosphorylation. *J Biochem Mol Biol* **40:** 432–438
Klein-Hitpass L, Ryffel GU, Heitlinger E, Cato AC (1988) A 13 bp palindrome is a functional estrogen responsive element and interacts specifically with estrogen receptor. *Nucleic Acids Res* **16:** 647–663
Kysela B, Chovanec M, Jeggo PA (2005) Phosphorylation of linker histones by DNA-dependent protein kinase is required for DNA ligase IV-dependent ligation in the presence of histone H1. *Proc Natl Acad Sci USA* **102:** 1877–1882
Lees-Miller SP (1996) The DNA-dependent protein kinase, DNA-PK: 10 years and no ends in sight. *Biochem Cell Biol* **74:** 503–512
Li J, Stern DF (2005) Regulation of CHK2 by DNA-dependent protein kinase. *J Biol Chem* **280:** 12041–12050
Martinez E, Wahli W (1989) Cooperative binding of estrogen receptor to imperfect estrogen-responsive DNA elements correlates with their synergistic hormone-dependent enhancer activity. *EMBO J* **8:** 3781–3791
Matsuoka S, Rotman G, Ogawa A, Shiloh Y, Tamai K, Elledge SJ (2000) Ataxia telangiectasia-mutated phosphorylates Chk2 *in vivo* and *in vitro*. *Proc Natl Acad Sci USA* **97:** 10389–10394
Medunjanin S, Hermani A, De SB, Grisouard J, Rincke G, Mayer D (2005) Glycogen synthase kinase-3 interacts with and phosphorylates estrogen receptor-α and is involved in the regulation of receptor activity. *J Biol Chem* **280:** 33006–33014
Norbury CJ, Hickson ID (2001) Cellular responses to DNA damage. *Annu Rev Pharmacol Toxicol* **41:** 367–401
Rothkamm K, Lobrich M (2003) Evidence for a lack of DNA double-strand break repair in human cells exposed to very low X-ray doses. *Proc Natl Acad Sci USA* **100:** 5057–5062
Ruiz C *et al* (2006) Tissue microarrays for comparing molecular features with proliferation activity in breast cancer. *Int J Cancer* **118:** 2190–2194
Sedelnikova OA, Rogakou EP, Panyutin IG, Bonner WM (2002) Quantitative detection of (125)IdU-induced DNA double-strand breaks with γ-H2AX antibody. *Radiat Res* **158:** 486–492
Shen H, Schultz M, Kruh GD, Tew KD (1998) Increased expression of DNA-dependent protein kinase confers resistance to adriamycin. *Biochim Biophys Acta* **1381:** 131–138
Smith GC, Jackson SP (1999) The DNA-dependent protein kinase. *Genes Dev* **13:** 916–934
Wang XQ, Redpath JL, Fan ST, Stanbridge EJ (2006) ATR dependent activation of Chk2. *J Cell Physiol* **208:** 613–619
Wazer DE, Tercilla OF, Lin PS, Schmidt-Ullrich R (1989) Modulation in the radiosensitivity of MCF-7 human breast carcinoma cells by 17β-estradiol and tamoxifen. *Br J Radiol* **62:** 1079–1083
Wei X, Xu H, Kufe D (2006) MUC1 oncoprotein stabilizes and activates estrogen receptor-α. *Mol Cell* **21:** 295–305
Woods T, Wang W, Convery E, Errami A, Zdzienicka MZ, Meek K (2002) A single amino acid substitution in DNA-PKcs explains the novel phenotype of the CHO mutant, XR-C2. *Nucleic Acids Res* **30:** 5120–5128