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Ionizing Radiation Inhibits the PLK Cell Cycle Gene in a G_2 Checkpoint-dependent Manner

ANNE HANSEN REE[1,2], ASE BRATLAND[1,2], KIRSTI SOLBERG LANDSVERK[3] and ØYSTEIN FODSTAD[1]

Departments of [1]Tumor Biology, [2]Oncology and [3]Biophysics, The Norwegian Radium Hospital, 0310 Oslo, Norway

Abstract. *Tumor cell cycle arrest at the cell cycle G_2/M boundary after ionizing radiation involves inhibition of the Polo-like kinase 1 (Plk1). We recently found that the mechanism comprised repression of its gene, PLK, mediated by the tumor-suppressor protein BRCA1. In the present study we examined the regulatory responses on PLK and cell cycle phases in breast carcinoma cell lines exposed to various modes of therapeutic irradiation. The tumor cells, harboring different DNA damage checkpoint defects, were irradiated with either a single dose of 8.0 Gy or fractionated doses accumulating to 8.0 Gy. In the BRCA1$^{-/-}$ HCC1937 cell line both radiation regimens caused moderate repression of PLK mRNA expression, whereas the reconstituted wild-type (wt) BRCA1 genotype of the HCC1937/BRCA1wt cell line was associated with significant down-regulation of PLK mRNA expression after irradiation. In contrast to the HCC1937 cell lines, the MCF7/LCC2 cells displayed the characteristic wt TP53 constitution of persistent, radiation-induced CDKN1A mRNA expression (encoding the G_1 cell cycle inhibitor p21$^{Waf1/Cip1/Sdi1}$). The regulatory effects on PLK in the MCF7/LCC2 cells, however, were identical to those in the HCC1937/BRCA1wt cell line. Moreover, whereas neither HCC1937 cell line displayed G_1/S cell cycle arrest after irradiation but, instead, an apparent accumulation of G_2/M-phase cells, the radiation-induced delay at the G_1/S boundary seemed to be superior to arrest at the G_2/M transition in the MCF7/LCC2 cell line. Since the down-regulation of PLK mRNA expression by ionizing radiation was identical in the wt TP53 MCF7/LCC2 cell line and the TP53-mutated BRCA1$^{-/-}$ HCC1937 cell line reconstituted with wt BRCA1, we conclude that this regulatory effect solely requires an intact G_2 checkpoint effector mechanism.*

Correspondence to: Anne Hansen Ree, Department of Tumor Biology, The Norwegian Radium Hospital, 0310 Oslo, Norway. Tel: +47 22-935421, Fax: +47 22-522421, e-mail: a.h.ree@labmed.uio.no

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Cell cycle checkpoints are surveillance systems that ensure orderly and timely replication and segregation of the genome. The tumor cell response to the DNA damage induced by ionizing radiation in cancer therapy involves activation of the cell cycle G_2 checkpoint to block mitotic entry until the double-strand DNA break is repaired. In clinical radiation therapy a minimal tumor cell delay in G_2-phase is desirable to limit the probability of this repair for cell death to succeed during mitosis.

In the orderly dividing cell the G_2/M transition is inhibited through phosphorylations of the Cdc2 kinase of the Cdc2/cyclin B complex. Upon the onset of mitosis these inhibitory phosphorylations are removed by Cdc25C phosphatase. The activation of Cdc25C requires positive regulatory phosphorylation, accomplished by Plk1 (1, 2), which should possibly be considered the mitotic trigger kinase. Plk1 is an evolutionary conserved serine/threonine kinase that is also involved in other stages throughout mitotic progression, such as centrosome maturation and bipolar spindle formation, as well as regulation of the anaphase-promoting complex/cyclosome and cytokinesis (3).

DNA damage activates G_2 checkpoint ATM (for 'ataxia telangiectasia mutated') kinase signaling (4), which is communicated through the downstream kinases CHEK1 and CHEK2 (5-7). This disrupts the interaction of Cdc25C with Cdc2 (7-10). Moreover, the enzymatic activity of Plk1 is also inhibited following DNA damage (11, 12). We have recently suggested that the regulatory effect of ionizing radiation comprises a repression mechanism on the gene for Plk1, PLK, mediated by the tumor-suppressor protein BRCA1 (13).

The BRCA1 gene encodes a 1863 residue nuclear protein that exerts its function in several fundamental processes, including the maintenance of genomic integrity, transcriptional gene regulation and cell cycle control (14, 15). In response to ionizing radiation, BRCA1 contributes as mediator of the activated ATM kinase signaling pathway (5, 16-18), and the BRCA1 activation seems to implicate both CHEK1 and CHEK2 (6, 19).

Whereas the identity of the putative upstream kinase(s) that is directly responsible for activation of Plk1 has not yet been revealed (20, 21), cell cycle-regulated repressor elements in the PLK promoter are characterized (22, 23). Consequently, in the present study we explored the requirement of intact cell cycle checkpoints for radiation-induced PLK repression and further compared the regulatory effects of fractionated *versus* single-dose exposure to ionizing radiation. We used the human BRCA1$^{-/-}$ HCC1937 cell line (24), in which TP53 is mutated (24) and G_2 checkpoint signaling *via* CHEK1 is abrogated (6), and two derivative cell lines. These represent the parental HCC1937 cells stably infected with viruses encoding wt BRCA1 (the HCC1937/BRCA1wt cells) and the vector carrying a green fluorescence protein (GFP) but no BRCA1 allele (the HCC1937/GFP cells), respectively (25). In addition, the human MCF7/LCC2 cells, harboring an intact cell cycle G_1 checkpoint due to their wt TP53 (26), were analyzed.

Materials and Methods

Cell cultures. The HCC1937 cell line (24) was obtained from the American Type Culture Collection (Manassas, VA, USA), whereas the retrovirally infected HCC1937/BRCA1wt and HCC1937/GFP cell lines (25) were kindly provided by Dr. David M. Livingston (Dana Farber Cancer Institute, Boston, MA, USA). The efficiency of plasmid uptake and the resulting full-length BRCA1 protein expression in the HCC1937/BRCA1wt cell line have been validated in the reference report (25). Moreover, we assayed emission of green fluorescence before the infected cell lines were used in experiments. The HCC1937 cell lines were routinely grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and 2.0 mM glutamine. The growth condition of the MCF7/LCC2 cells was described previously (26, 27). For all cell lines the growth media were replaced 24 h before start of the experiments by media containing 5% fetal bovine serum and glutamine only, and these experimental media were again changed at time 0.

Experimental irradiation. High-energy radiation from a ^{60}Co source was delivered at a rate of approximately 1.0 Gy/min. The cells were irradiated with either a single dose of 8.0 Gy or fractionated doses of 2.0 Gy given every 3 h until an accumulated dose of 8.0 Gy had been applied. The unirradiated control cells were simultaneously placed at room temperature to obtain exactly comparable conditions.

Northern blot analysis. Total RNA was extracted and analyzed by standard Northern blotting techniques. A human cDNA clone for PLK was obtained from RZPD Deutsches Ressourcenzentrum für Genomforschung GmbH (Berlin, Germany), whereas the human cDNA probe for CDKN1A was a gift from Dr. Bert Vogelstein (John Hopkins University School of Medicine, Baltimore, MD, USA). The Northern blot membranes were also hybridized to an oligonucleotide probe complementary to nucleotides 287-305 of human 18S rRNA. Finally, the autoradiograms were subjected to densitometric measurements in a laser densitometer and the mRNA expression levels relative to 18S rRNA were calculated.

Flow cytometry analysis. Cells were harvested by trypsinization and resuspended in ice-cold PBS. After centrifugation, the cell pellets were fixed and stored in 100% methanol at -20˚C. The cells were subsequently stained with 1.5 μg/ml Hoechst 33258 in PBS and analyzed in a FACStar+ flow cytometer (Becton Dickinson, San Jose, CA, USA). Forward scatter and side scatter were excited with 200 mW of 488 nm laser light (argon laser; Spectra Physics, Mountain View, CA, USA). Hoechst 33258 fluorescence (400-450 nm) was excited with 50 mW of UV light (351/356 nm) at a second laser intercept (krypton laser; Spectra Physics). The acquisition was triggered by the forward scatter signal. Doublets were discriminated from the analysis by using the area and width of the Hoechst 33258 signal. The Modfit cell cycle program (Verity Software House, Inc., Topsham, ME, USA) was used to determine the fractions of cells in the G_1-, S-, and G_2/M-phases from the cell cycle distribution.

Results

Cell cycle checkpoints and regulation by ionizing radiation of PLK mRNA. To analyze the radiation-induced PLK repression (13) in detail, the breast carcinoma cell lines, harboring different DNA damage checkpoint defects, were irradiated with four consecutive, fractionated doses of 2.0 Gy or a single dose of 8.0 Gy. The mRNA expression levels of PLK as well as CDKN1A (encoding the G_1 cell cycle inhibitor p21$^{Waf1/Cip1/Sdi1}$) were followed every 3 h for a total time period of 12 h.

Treatment of the BRCA1$^{-/-}$ HCC1937 cells, which should be regarded partially G_2 checkpoint-defective (6), with ionizing radiation of the hyperfractionated as well as the single-dose regimen, was associated with a moderate repression of PLK mRNA expression (to 70-80% of the control level) throughout the entire incubation period following irradiation (Figures 1 and 2a). Upon reconstitution of an intact BRCA1 effector mechanism in the retrovirally-infected HCC1937/BRCA1wt cell line (25), a significant down-regulation of PLK mRNA expression was observed (Figure 1). The expression level was ~15% of control 6 h after exposure to the 8.0 Gy single-dose radiation but recovered to ~60% of control after 12 h. In contrast to this transient response, the hyperfractionated radiation regimen in the HCC1937/BRCA1wt cells resulted in an invariably repressed level of PLK mRNA expression at 45-50% of control throughout the treatment period of 12 h (Figure 2b). As regards the HCC1937/GFP control-infected cells, the radiation responses on PLK mRNA were closely similar to the expression patterns observed in the HCC1937 parental cell line (results not shown).

The MCF7/LCC2 cells were exposed to the same regimens of ionizing radiation to examine whether the cell cycle G_1 checkpoint might also contribute to the radiation-dependent responses of PLK mRNA. As seen from Figure 1, this cell line was confirmed to harbor intact p53 checkpoint characteristics, as both the hyperfractionated and

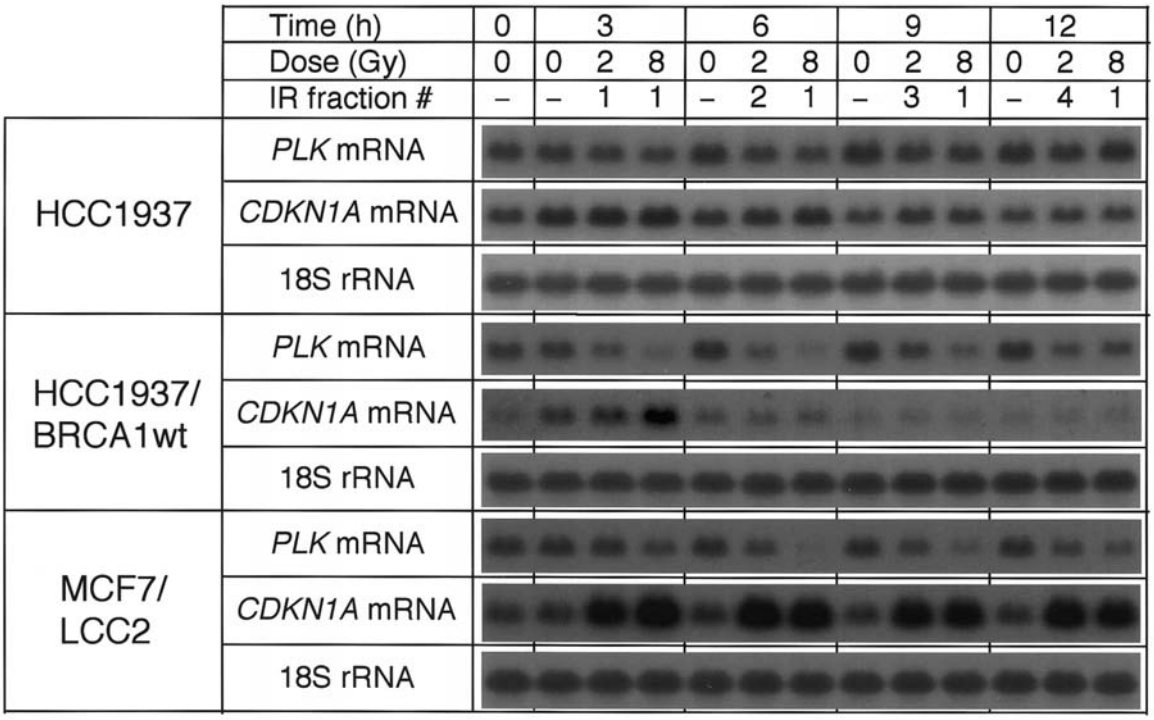

Figure 1. *Expression of mRNAs for PLK and CDKN1A upon various modes of irradiation. The HCC1937, HCC1937/BRCA1wt and MCF7/LCC2 cell lines were treated as indicated: unirradiated (0 Gy), or exposed to ionizing radiation (IR) either repeatedly (1-4 fractions) with doses of 2.0 Gy every 3 h from time 0 until an accumulated dose of 8.0 Gy had been applied (hyperfractionated treatment) or with a single-dose of 8.0 Gy at time 0. Expression of mRNAs for PLK and CDKN1A after 0-12 h was determined by Northern blot analysis. 18S rRNA was measured as RNA loading control.*

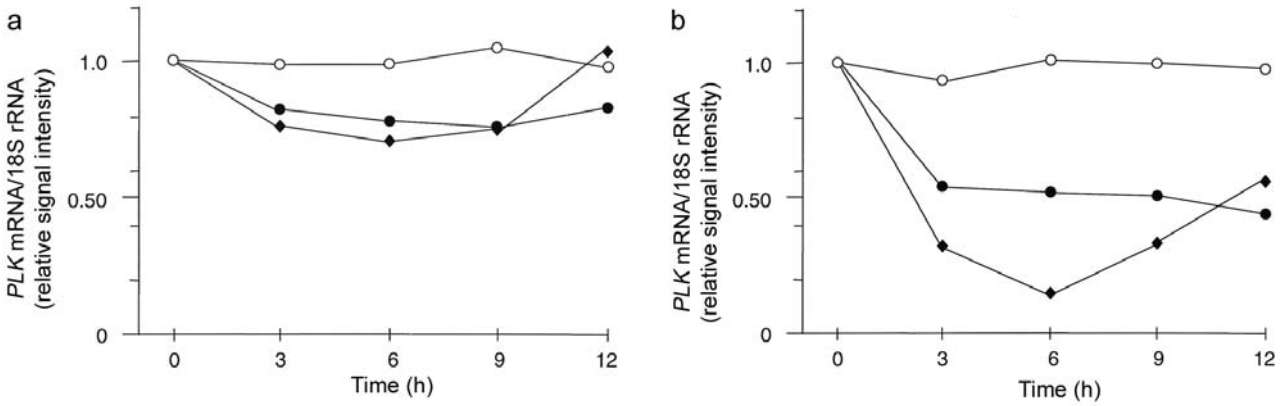

Figure 2. *Time course of PLK down-regulation after irradiation as function of the BRCA1 signaling pathway. The plots represent densitometric calculations of the PLK mRNA/18S rRNA signals, displayed in Figure 1, obtained from the BRCA1$^{-/-}$ HCC1937 (a) and HCC1937/BRCA1wt (b) cell lines that were either unirradiated controls (○) or were exposed to the hyperfractionated (●) or the single-dose (◆) treatment. Values are plotted relative to the time 0 value for each cell line.*

single-dose treatment regimens caused striking up-regulation of the mRNA for CDKN1A, reaching a steady-state level of >5-fold above control probably before the first time-point (3 h) was measured. The regulatory effects on PLK in the MCF7/LCC2 cells, however, were principally similar to those in the HCC1937/BRCA1wt cell line. Interestingly, whereas the baseline mRNA level for CDKN1A in the parental HCC1937 cells was principally unaffected by exposure to ionizing radiation, as expected when TP53 is mutated, the hardly detectable baseline expression of this mRNA in the

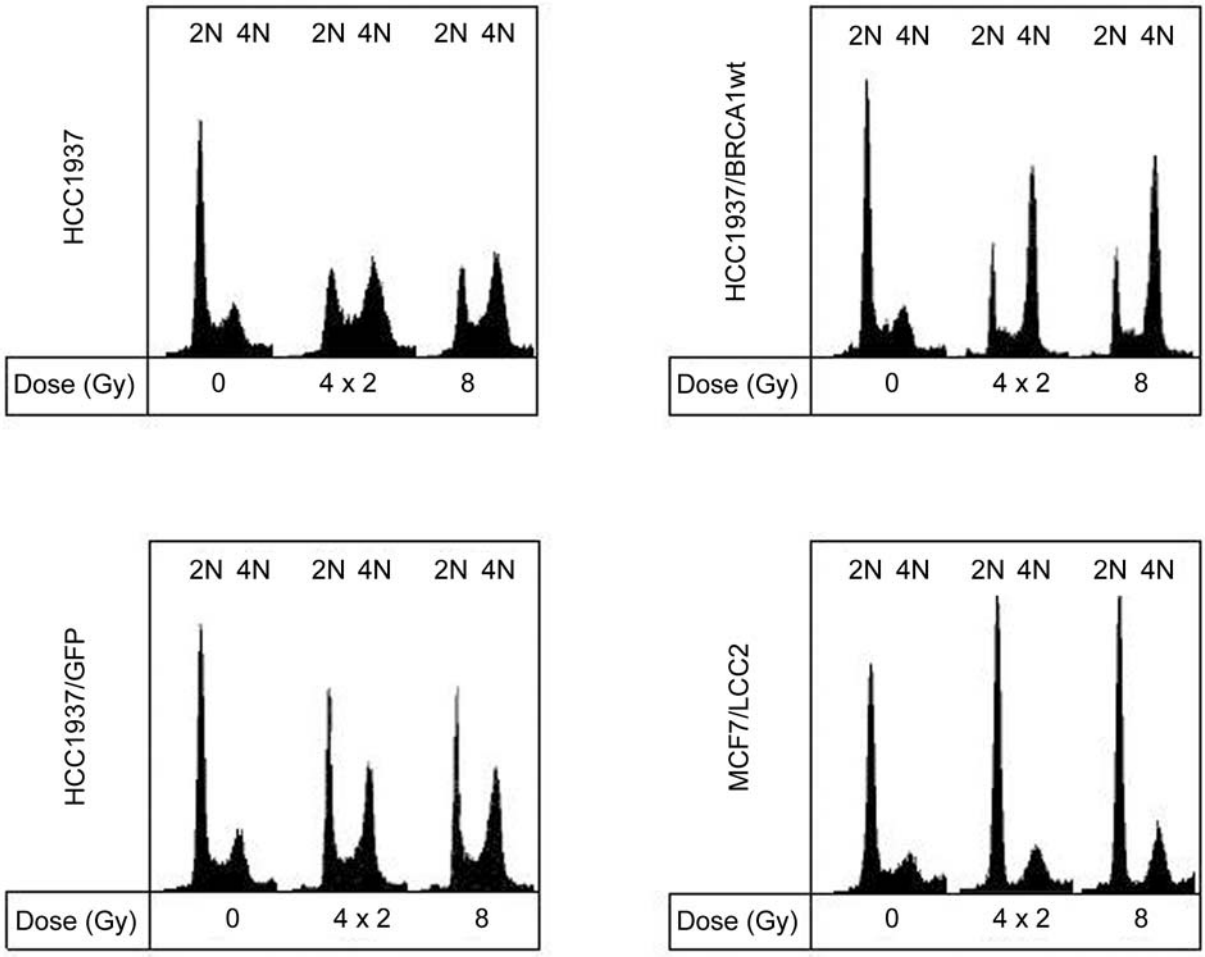

Figure 3. *Cell cycle profiles of irradiated breast carcinoma cell lines harboring different DNA damage checkpoint defects. The HCC1937, HCC1937/BRCA1wt, HCC1937/GFP and MCF7/LCC2 cell lines were left unirradiated (0 Gy), or exposed to ionizing radiation either repeatedly with doses of 2.0 Gy every 3 h from time 0 until an accumulated dose of 8.0 Gy had been applied (4 x 2.0 Gy) or with an equivalent single-dose (8.0 Gy) at time 0. Cell cycle profiles were determined after 24 h by FACS® analysis. The peaks labeled 2N and 4N represent cells with DNA contents characteristic for the G1- and G2/M-phases of the cell cycle, respectively.*

derivative HCC1937/BRCA1wt cells showed a significant up-regulation after 3 h, followed by an abrupt decline back to the baseline level (Figure 1).

Redistribution of cell cycle phases after exposure to ionizing radiation. Whether cell cycle profiles might reflect the PLK mRNA responses to hyperfractionated and single-dose irradiation was analyzed in cells given the treatment regimens described above. The HCC1937 parental and derivative cell lines displayed complete absence of wt TP53-dependent accumulation of G_1-phase cells after the DNA damage (Figure 3). Radiation-induced G_2/M-phase arrest of the HCC1937 cells emerged after 9 h, irrespective of the mode of fractionation (Figure 4a), whereas the HCC1937/BRCA1wt cell line showed a more immediate arrest at the G_2/M boundary, already measurable 3-6 h after radiation exposure (Figure 4b). Furthermore, the rate of accumulation of the G_2/M cell fractions was slower in the parental than in the derivative cell line. The fractions of HCC1937 cells that arrested in the G_2/M-phase were ~55% 48 h after exposure to both hyperfractionated and single-dose radiation regimens (Figure 4a), whereas the corresponding values for the HCC1937/BRCA1wt cell line were significantly higher, namely ~85 and ~90%, respectively (Figure 4b). The redistribution of cell cycle phases in irradiated HCC1937/GFP control-infected cells

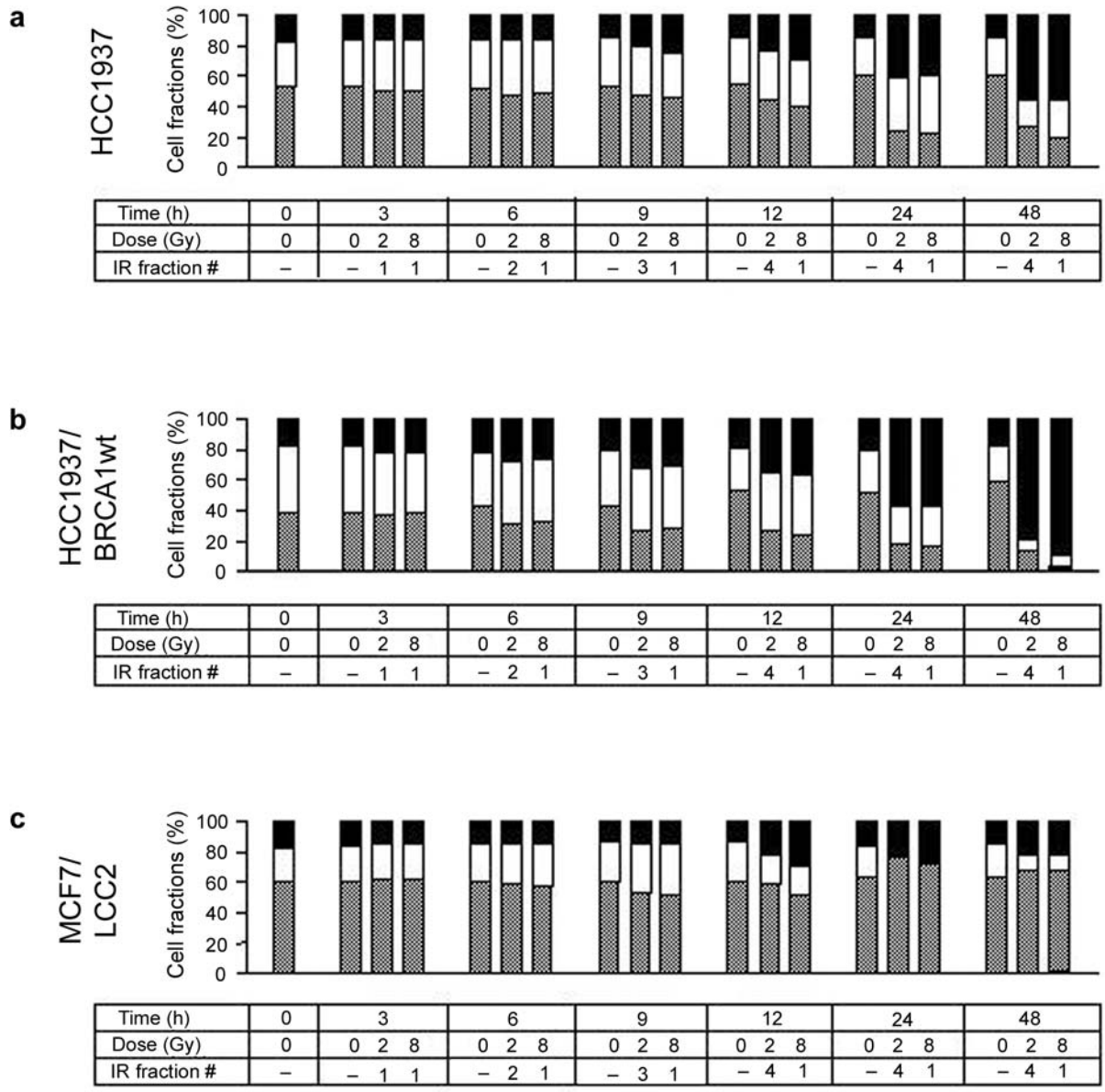

Figure 4. *Redistribution of cell cycle phases upon various modes of irradiation. The HCC1937 (a), HCC1937/BRCA1wt (b) and MCF7/LCC2 (c) cell lines were treated as indicated: unirradiated (0 Gy), or exposed to ionizing radiation (IR) either repeatedly (1-4 fractions) with doses of 2.0 Gy every 3h from time 0 until an accumulated dose of 8.0 Gy had been applied or with a single-dose of 8.0 Gy at time 0. Cellular DNA contents after 0-48 h were determined by FACS® analysis. The distribution of cell cycle phases is displayed as columns representing the phase-specific percentages of each total cell counts: G1-phase cells (shaded boxes) S-phase cells (white boxes), and G2/M-phase cells (black boxes).*

resembled what was observed in the HCC1937 parental cell line (Figure 3).

Compared to the HCC1937 cell lines, accumulation in the G_2/M-phase was a late event in irradiated MCF7/LCC2 cells (Figures 3 and 4c). No evidence of G_2/M-phase arrest was observed earlier than 12 h after the start of radiation, when the fractions of cells accumulating at the G_2/M boundary had increased from <15% in unirradiated cells to >20 and ~30%, respectively, in cells exposed to the hyperfractionated and single-dose regimens. Each of these radiation responses was preceded by a transient accumulation of S-phase cells. Moreover, specific accumulation of G_1-phase cells emerged at 12 h and was apparent 24 h after the start of irradiation. In both treatment regimens, residual arrest of cells in both G_1- and G_2/M-phases was still detectable after 48 h.

Discussion

DNA damage activates the G_2 cell cycle checkpoint to allow time for DNA repair before mitotic entry. We have recently found that tumor cell cycle arrest at the G_2/M boundary after exposure to ionizing radiation may involve repression of PLK gene expression, mediated by the tumor-suppressor protein BRCA1 (13). In the present study, we further explored the requirement of intact cell cycle checkpoints for the radiation-induced PLK repression and found that down-regulation of PLK mRNA expression was identical in the wt TP53 MCF7/LCC2 cell line and the TP53-mutated BRCA1$^{-/-}$ HCC1937 cell line reconstituted with wt BRCA1, indicating that this regulatory effect solely requires an intact G_2 checkpoint effector mechanism. The present results also demonstrated that the kinetics of these BRCA1-mediated responses seemed to depend on the fractionation of the applied radiation dose.

The HCC1937 cell line is hemizygous with respect to the BRCA1 disease-mutated allele 5382insC and synthesizes a truncated BRCA1 protein that lacks parts of the extreme C-terminal BRCT (for 'BRCA1 C-terminal') repeats (24, 25). The BRCT domains are shown to interact with a variety of transcriptional regulators (14), including the adaptor protein CtIP (28), which functions as a transcriptional co-repressor within histone deacetylase complexes (29). Tentatively, the DNA damage-activated, functional BRCA1 could facilitate recruitment of CtIP into a regulatory unit that inhibits transcriptional activity of the PLK gene promoter.

DNA damage inhibits the kinase activity of Plk1 (11, 12). Whereas the enzymatic activity reflects its phosphorylation status (20, 21), turnover of the kinase protein is regulated *via* ubiquitination (30). PLK has been shown to be among several genes, encoding mitotic regulators, of which the mRNA expression levels are down-regulated following activation of the G_2 cell cycle checkpoint (31). The promoter of human PLK contains a characteristic repressor element in the region of the transcription start site, mediating the cell cycle phase-specific regulation of the gene expression (22). This repressor element also contributes to inhibition of PLK transcription after activation of the cell cycle inhibitor p21$^{Waf1/Cip1/Sdi1}$ (23), which can function as a highly specific transcriptional regulator of numerous genes involved in cell cycle progression and DNA repair (32). Our demonstration in irradiated wt TP53 MCF7/LCC2 cells, revealing that the instant induction of CDKN1A mRNA clearly preceded the inhibitory effect on PLK mRNA, may suggest a role of p21$^{Waf1/Cip1/Sdi1}$ in radiation-directed regulation of PLK transcription. The observation that even the G_1 checkpoint-defective HCC1937/BRCA1wt cell line displayed CDKN1A mRNA induction, although transient, may also support this concept. However, the possibility that p21$^{Waf1/Cip1/Sdi1}$ is a mediator of PLK repression following ionizing radiation argues against the BRCA1 pathway as the principal effector mechanism.

The MCF7/LCC2 cell line harbored the characteristic wt TP53 constitution of radiation-induced CDKN1A mRNA expression. Also, the rapid but transient arrest in the cell cycle S-phase typically seen in cells with intact p53 effector pathway (18) was found. Indeed, the radiation-induced arrest at the G_1/S boundary seemed to be superior to the regulatory effect at the G_2/M transition in this cell line.

The mechanism of PLK mRNA down-regulation after exposure to ionizing radiation may principally comprise a decrease in mRNA stability, as has been suggested for other cell cycle genes (33, 34). Regarding the previously reported p21$^{Waf1/Cip1/Sdi1}$-mediated inhibition of PLK expression, however, this is claimed not to involve changes in mRNA half-life (23).

When adverse effects from the normal tissues that are included in the irradiated volume are at risk of exceeding a clinically tolerable level, radiation therapy is often applied as multiple, lower doses, in terms of a hyperfractionated regimen, to attenuate this toxicity. Whether regulatory mechanisms that are operative with hyperfractionated irradiation are different from those with single-dose treatment is still to be determined. Our cell cycle data interestingly indicated that, whereas the radiation response of the G_1-phase CDKN1A gene did not distinguish between different fractionation, the kinetics of the G_2-phase PLK responses to ionizing radiation clearly did. Principally, however, redistribution of cell cycle phases after radiation seemed to be independent of the type of dose fractionation applied.

Human Plk1 was originally identified on the basis of its high expression levels in rapidly proliferating cells (35). More recent data have suggested that elevated expression of this kinase in the primary tumor is associated with an unfavorable outcome for patients with head and neck carcinomas as well as malignant melanomas (36, 37). Moreover, experimental silencing of tumor cell Plk1 in model systems is shown to inhibit the proliferative capacity of the tumor cells (38, 39). In clinical radiation therapy the rapidly proliferating tumor cells are the more radiosensitive because a minimal tumor cell delay in the cell cycle G_2-phase will limit the probability of double-strand DNA break repair before mitotic entry. Our results, indicating that Plk1 inhibition in DNA damage checkpoint control (11, 12) principally comprises a gene repression mechanism, may suggest a therapeutic benefit of PLK gene replacement therapy or pharmaceutical targeting of this signaling pathway in tumor cell radiosensitization.

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References

1 Roshak AK, Capper EA, Imburgia C, Fornwald J, Scott G and Marshall LA: The human *polo*-like kinase, PLK, regulates cdc2/cyclin B through phosphorylation and activation of the cdc25C phosphatase. Cell Signal *12*: 405-411, 2000.
2 Toyoshima-Morimoto F, Taniguchi E and Nishida E: Plk1 promotes nuclear translocation of human Cdc25C during prophase. EMBO Rep *3*: 341-348, 2002.
3 Nigg EA: Mitotic kinases as regulators of cell division and its checkpoints. Nat Rev *2*: 21-32, 2001.
4 Bakkenist CJ and Kastan MB: DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature *421*: 499-506, 2003.
5 Cortez D, Wang Y, Qin J and Elledge SJ: Requirement of ATM-dependent phosphorylation of Brca1 in the DNA damage response to double-strand breaks. Science *286*: 1162-1166, 1999.
6 Yarden RI, Pardo-Reoyo S, Sgagias M, Cowan KH and Brody LC: BRCA1 regulates the G_2/M checkpoint by activating Chk1 kinase upon DNA damage. Nat Genet *30*: 285-289, 2002.
7 Matsuoka S, Huang M and Elledge SJ: Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science *282*: 1893-1897, 1998.
8 Furnari B, Rhind N and Russell P: Cdc25 mitotic inducer targeted by Chk1 DNA damage checkpoint kinase. Science *277*: 1495-1497, 1997.
9 Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnica-Worms H and Elledge SJ: Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Chk regulation through Cdc25. Science *277*: 1497-1501, 1997.
10 Lopez-Girona A, Furnari B, Mondesert O and Russell P: Nuclear localization of Cdc25 is regulated by DNA damage and a 14-3-3 protein. Nature *397*: 172-175, 1999.
11 Smits VAJ, Klompmaker R, Arnaud L, Rijksen G, Nigg EA and Medema RH: Polo-like kinase-1 is a target of the DNA damage checkpoint. Nat Cell Biol *2*: 672-676, 2000.
12 van Vugt MATM, Smits VAJ, Klompmaker R and Medema RH: Inhibition of polo-like kinase-1 by DNA damage occurs in an ATM- or ATR-dependent fashion. J Biol Chem *276*: 41656-41660, 2001.
13 Ree AH, Bratland A, Nome RV, Stokke T and Fodstad Ø: Repression of mRNA for the PLK cell cycle gene after DNA damage requires BRCA1. Oncogene *22*: 8952-8955, 2003.
14 Deng CX and Brodie SG: Roles of *BRCA1* and its interacting proteins. Bioessays *22*: 728-737, 2000.
15 Scully R and Livingston DM: In search of the tumour-suppressor functions of BRCA1 and BRCA2. Nature *408*: 429-432, 2000.
16 Li S, Ting NSY, Zheng L, Chen PL, Ziv Y, Shiloh Y, Lee EYHP and Lee WH: Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response. Nature *406*: 210-215, 2000.
17 Wu-Baer F and Baer R: Effect of DNA damage on a BRCA1 complex. Nature *414*: 36, 2001.
18 Xu B, Kim ST and Kastan MB: Involvement of Brca1 in S-phase and G_2-phase checkpoints after ionizing radiation. Mol Cell Biol *21*: 3445-3450, 2001.
19 Lee JS, Collins KM, Brown AL, Lee CH and Chung JH: hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. Nature *404*: 201-204, 2000.
20 Kelm O, Wind M, Lehmann WD and Nigg EA: Cell cycle-regulated phsphorylation of the *Xenopus* polo-like kinase Plx1. J Biol Chem *277*: 25247-25256, 2002.
21 Jang YJ, Ma S, Terada Y and Erikson RL: Phosphorylation of threonine 210 and the role of serine 137 in the regulation of mammalian polo-like kinase. J Biol Chem *277*: 44115-44120, 2002.
22 Uchiumi T, Longo DL and Ferris D: Cell cycle regulation of the human polo-like kinase (*PLK*) promoter. J Biol Chem *272*: 9166-9174, 1997.
23 Zhu H, Chang BD, Uchiumi T and Roninson IB: Identification of promoter elements responsible for transcriptional inhibition of polo-like kinase 1 and topoisomerase II alpha genes by $p21^{WAF1/CIP1/SDI1}$. Cell Cycle *1*: 59-66, 2002.
24 Tomlinson GE, Chen TTL, Stastny VA, Virmani AK, Spillman MA, Tonk V, Blum JL, Schneider NR, Wistuba II and Gazdar AF: Characterization of a breast cancer cell line derived from a germ-line BRCA1 mutation carrier. Cancer Res *58*: 3237-3242, 1998.
25 Scully R, Ganesan S, Vlasakova K, Chen J, Socolovsky M and Livingston DM: Genetic analysis of BRCA1 function in a defined tumor cell line. Mol Cell *4*: 1093-1099, 1999.
26 Brünner N, Frandsen TL, Holst-Hansen C, Bei M, Thompson EW, Wakeling AE, Lippman ME and Clarke R: MCF7/LCC2: a 4-hydroxytamoxifen resistant human breast cancer variant that retains sensitivity to the steroidal antiestrogen ICI 182,780. Cancer Res *53*: 3229-3232, 1993.
27 Ree AH, Tvermyr M, Engebraaten O, Rooman M, Røsok Ø, Hovig E, Meza-Zepeda LA, Bruland ØS and Fodstad Ø: Expression of a novel factor in human breast cancer cells with metastatic potential. Cancer Res *59*: 4675-4680, 1999.
28 Yu X, Wu LC, Bowcock AM, Aronheim A and Baer R: The C-terminal (BRCT) domains of BRCA1 interact *in vivo* with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. J Biol Chem *273*: 25388-25392, 1998.
29 Chinnadurai G: CtBP, an unconventional transcriptional corepressor in development and oncogenesis. Mol Cell *9*: 213-224, 2002.
30 Kang D, Chen J, Wong J and Fang G: The checkpoint protein Chfr is a ligase that ubiquitinates Plk1 and inhibits Cdc2 at the G_2 to M transition. J Cell Biol *156*: 249-259, 2002.
31 Crawford DF and Piwnica-Worms H: The G_2 DNA damage checkpoint delays expression of genes encoding mitotic regulators. J Biol Chem *276*: 37166-37177, 2001.
32 Chang BD, Watanabe K, Broude EV, Fang J, Poole JC, Kalinichenko TV and Roninson IB: Effects of $p21^{Waf1/Cip1/Sdi1}$ on cellular gene expression: implications for carcinogenesis, senescence, and age-related diseases. Proc Natl Acad Sci USA *97*: 4291-4296, 2000.
33 Datta R, Hass R, Gunji H, Weichselbaum R and Kufe D: Down-regulation of cell cycle control genes by ionizing radiation. Cell Growth Differ *3*: 637-644, 1992.

34 Maity A, McKenna WG and Muschel RJ: Evidence for post-transcriptional regulation of cyclin B1 mRNA in the cell cycle and following irradiation in HeLa cells. EMBO J *14*: 603-609, 1995.

35 Holtrich U, Wolf G, Bräuninger A, Karn T, Böhme B, Rübsamen-Waigmann H and Strebhardt K: Induction and down-regulation of PLK, a human serine/threonine kinase expressed in proliferating cells and tumors. Proc Natl Acad Sci USA *91*: 1736-1740, 1994.

36 Knecht R, Elez R, Oechler M, Solbach C, von Ilberg C and Strebhardt K: Prognostic significance of *Polo-like kinase* (*PLK*) expression in squamous cell carcinomas of the head and neck. Cancer Res *59*: 2794-2797, 1999.

37 Kneisel L, Strebhardt K, Bernd A, Wolter M, Binder A and Kaufmann R: Expression of polo-like kinase (PLK1) in thin melanomas: a novel marker of metastatic disease. J Cutan Pathol *29*: 354-358, 2002.

38 Spänkuch-Schmitt B, Bereiter-Hahn J, Kaufmann M and Strebhardt K: Effect of RNA silencing of polo-like kinase-1 (PLK1) on apoptosis and spindle formation in human cancer cells. J Natl Cancer Inst *94*: 1863-1877, 2002.

39 Elez R, Piiper A, Kronenberger B, Kock M, Brendel M, Hermann E, Pliquett U, Neumann E and Zeuzem S: Tumor regression by combination antisense therapy against Plk1 and Bcl-2. Oncogene *22*: 69-80, 2003.

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