DNA Damage Recognition via Activated ATM and p53
Pathway in Nonproliferating Human Prostate Tissue

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Abstract
DNA damage response (DDR) pathways have been extensively studied in cancer cell lines and mouse models, but little is known about how DNA damage is recognized by different cell types in nonmalignant, slowly replicating human tissues. Here, we assess, using ex vivo cultures of human prostate tissue, DDR caused by cytotoxic drugs (camptothecin, doxorubicin, etoposide, and cisplatin) and ionizing radiation (IR) in the context of normal tissue architecture. Using specific markers for basal and luminal epithelial cells, we determine and quantify cell compartment-specific damage recognition. IR, doxorubicin, and etoposide induced the phosphorylation of H2AX on Ser139 (γH2AX) and DNA damage foci formation. Surprisingly, luminal epithelial cells lack the prominent γH2AX response after IR when compared with basal cells, although ATM phosphorylation on Ser1981 and 53BP1 foci were clearly detectable in both cell types. The attenuated γH2AX response seems to result from low levels of total H2AX in the luminal cells. Marked increase in p53, a downstream target of the activated ATM pathway, was detected only in response to camptothecin and doxorubicin. These findings emphasize the diversity of pathways activated by DNA damage in slowly replicating tissues and reveal an unexpected deviation in the prostate luminal compartment that may be relevant in prostate tumorigenesis. Detailed mapping of tissue and cell type differences in DDR will provide an outlook of relevant responses to therapeutic strategies. Cancer Res; 70(21); 8630–41. ©2010 AACR.

Introduction
DNA damage response (DDR) pathways protect genomic integrity and form a barrier against cancer (1). DDR includes multiple mechanisms that detect damaged DNA, activate the repair machineries, delay cell cycle progression, and cause permanent replicative arrest or activate apoptosis (2). The nature and amount of DNA damage, tissue-specific factors, and developmental stage determine types of pathways that are activated (3–5). It is not known why some tissue and cell types are more prone to tumorigenesis and why inactivation of central DDR pathways leads to cancer in only certain tissues (6, 7). To better understand how DDR participates in human disease, it will be useful to delineate which signaling molecules participate in the responses in different tissues. Cell-based assays have been most useful in studying how genomic integrity is maintained, but they do not provide information on how heterotopic interactions or three-dimensional tissue environment affect DDR. Equally, despite that tumor suppressor and DDR pathways are generally conserved in mammals, mouse models do not recapitulate all aspects of DNA damage surveillance in human cells or tissues (8).

DNA double-strand breaks (DSB) are considered most detrimental for genomic integrity, because their unsuccessful repair can lead to chromosomal aberrations. After ionizing radiation (IR), the DSB recognition and modification of exposed chromatin occurs within minutes, and one of the early sensors, activated phosphoinositide 3-kinase (PI3K) ATM [Ser1981 phosphorylated ATM (pATM)] is recruited to the lesion by the Mre11-Rad50-Nbs1 (MRN) complex (9, 10). ATM, its homologue ATR, and DNA-dependent protein kinase (DNA-PK) are considered the primary kinases redundantly phosphorylating histone variant H2AX at its Ser139 residue after DSB and other types of DNA damage stress in mouse and human cells (11, 12). Tissue specificity between the kinases has been shown to exist in vivo (13, 14). γH2AX is...
one of the first proteins accumulating at DSB (15, 16) and is considered essential for the maintenance of genomic integrity (17, 18). γH2AX has been shown to be dispensable for the initial recognition of DNA breaks in murine embryo fibroblasts (19) but is required for the retention of several DDR proteins, such as 53BP1, BRCA1, and MDC1, at DNA damage foci (17, 18, 20). Together with MDC1, γH2AX forms a platform onto which other DDR proteins, such as MRN-ATM complex, 53BP1, and BRCA1, attach (11). The composition of DNA repair proteins at the damage site may vary and affect which DDR pathway is activated (21).

p53 tumor suppressor protein regulates key steps in cell cycle checkpoint activation, apoptosis, DNA repair, and senescence and is frequently mutated in most cancer types (22). In response to IR, ATM phosphorylates p53 on Ser15, and p53 is stabilized at least in radiosensitive tissues like thymus and spleen (5, 23). However, ATM is not required for p53 induction in small intestine or epithelium (13), and we recently reported that human primary prostate epithelial cells show attenuated p53 response to IR (24). In agreement, previous studies have shown that primary cultures of human mammary epithelial cells, bronchial epithelial cells, and human keratinocytes also exhibit a differential p53 IR response (25). These findings indicate that mechanisms may have evolved to maintain p53 under tight control in epithelial tissues.

In this study, we investigate how human prostate tissue responds to DNA damage caused by chemotherapeutic drugs and IR. We show that p53 is stabilized by topoisomerase poisons, proteasomal and transcriptional stress in epithelium, also without prior activation of ATM pathway, and that IR and topoisomerase II poisons induce γH2AX foci and activate ATM in nonreplicating prostate cells. Surprisingly, compared with basal cells after DNA damage, luminal epithelial cells show negligible levels of γH2AX foci, although pATM and 53BP1 foci are clearly detectable in both cell types. Given that H2AX is required for efficient DNA DSB repair and the lack of it leads to chromosomal instability (11, 17, 18), it is possible that luminal epithelial cells are more prone to accumulating DNA lesions.

Materials and Methods

Ex vivo tissue culture and treatments
Prostate tissue was collected from 18 patients undergoing cystectomy or prostatectomy at Helsinki University Central Hospital or at Johns Hopkins Medicine during 2007 to 2009 with informed written consent of the patients and approval by institutional review boards (390/E6/06, 371/E6/07, and NA_00015481). Clinical data are presented in Supplementary Table S1. A cylinder of prostate tissue (8-mm diameter) was cored out of the peripheral dorsal region of the prostate, and the cylinder was sliced at 300- to 500-μm thickness using Krumdieck tissue slicer (Alabama Research and Development Corp.). The slices were placed onto titanium grids and cultured in serum-free RPMF-4A tissue culture medium supplemented with growth factors and androgen analogue R1881 (20 nmol/L) and incubated in a rotating platform at +37°C and 5% CO2 (24). The histopathology of H&E-stained specimens was evaluated by A.S., and only nonmalignant tissue slices were included in the study. All experimental treatments of the cultures were performed on days 1 to 2.

Doxorubicin, etoposide, camptothecin, cisplatin (Sigma-Aldrich), and MG132 (Biomol International LP) were diluted into DMSO, aliquoted, and stored at −20°C. Actinomycin D was purchased from Sigma, diluted into ethanol, aliquoted, and stored at −20°C. Tissues were irradiated with calibrated 137Cs γ-ray irradiator (BioBeam 8000, STS).

Immunohistochemistry
Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut to 4 μm. The sections were deparaffinized and rehydrated, and antigens were retrieved by heating the slides in a microwave oven (700–800 W) in 0.01 mol/L sodium citrate (pH 6.0) or Tris-EDTA (pH 9.0) buffer up to 20 minutes. Sections were incubated overnight at +4°C with the following primary antibodies: p53, DO-7 (1:50, Dako) and 7F5 (1:50, Cell Signaling Technology); γH2AX, clone JBW301 (1:800, Upstate Biotechnology), 20E3 (1:50, Cell Signaling Technology), and pSer1981 pATM (1:100, Cell Signaling); histone H2AX (1:200, Novus Biologicals and 1:50, Cell Signaling Technology); p21 Waf1/Cip1 (1:200, Novus Biologicals and 1:50, Cell Signaling Technology); p27 Kip1 (1:100, BD Biosciences and 1:200, Epitomics); p63 Ab-4 (1:100, Neomarkers); cytokeratin 5 (CK5) and CK14 (1:100, incubated 1 hour at room temperature (RT), ImageX); androgen receptor (AR; 1:50, Biocare Medical); α-smooth muscle actin (α-SMA) (1:1,000, Sigma-Aldrich); K67, MB-1 (1:100, Dako); 5-bromo-2-deoxyuridine (5-BrdUrd; 1:100, Sigma); 53BP1 (1:200, Novus Biologicals); pSer1981 pATM (1:100, Cell Signaling); and cleaved caspase-3 (Asp175) 5A1E (1:100, Cell Signaling). Secondary fluorescent antibodies, antimouse- or antirabbit-conjugated Alexa-488 or Alexa-594, were purchased from Molecular Probes (1:100) and incubated at RT for 30 minutes. Secondary biotinylated antimouse or antirabbit antibodies (1:100, Dako) were incubated at RT for 30 minutes each. The tissues were counterstained with Hoechst 33342 (1 μg/mL; Molecular Probes) or with hematoxylin (Mayers hemalum solution, Merck KGaA) and mounted with Vectashield (Vector Laboratories) or GVA mount (Invitrogen).

Image acquisition and analysis
Transmitted light images were captured with Leica DM LB research microscope using N Plan objectives, Olympus DP50 color camera, and StudioLite 1.0 software. Fluorescence images were acquired with Zeiss Axioplan 2 MOT epifluorescence microscope, Zeiss Plan-Neofluar objectives, Zeiss AxioCam HRm 14-bit grayscale charge-coupled device camera and Zeiss Axiovision 4.6 software. Confocal images were single sections acquired sequentially using Zeiss LSM 510 META confocal microscope, 40× Plan-Neofluar DIC and 63× Plan-Apochromat DIC oil objectives, LSM 510 point scanner, Diode 405 laser for Hoechst, Argon 488 laser for Alexa-488 and HeNe1 543 laser for Alexa-594 label with appropriate filters, and LSM software release 3.2. The optical sections for 40× and 63× images were <1 μm.
For quantification, two to six fields of each specimen were captured using 40× objective on the wide-field epifluorescence microscope. The total number of cells analyzed for each treatment was on average 2,600. Image analysis was performed using a Mathworks Matlab-based application developed for the purpose and was designated as Cell Image Segmentation and Classifier (CISC). CISC was set to detect cell nuclei by thresholding DNA staining at several intensities and searching for shapes fitting the expectations of nuclear area and roundness (Supplementary Fig. S1A). Roundness is expressed as the ratio \((4\cdot\text{area})/(\text{perimeter length})^2\). Thresholding was set to identify objects of areas between 21.5 and 86.0 \(\mu\text{m}^2\) and roundness of >0.5.

The intensity of each protein was expressed as mean and SD. Margination was used for the outlier analysis. For nuclear proteins, margination was defined as the ratio of mean intensities in the center of the nucleus and at the borders of the nucleus. The border width was defined as 30% of the width of the nucleus in question.

Signal intensities were classified using \(k\)-means algorithm. Based on initial visual observation, we expected to retain four different intensity classes for \(\gamma\text{H2AX}\) in ascending order of mean intensities: negative, weak positive, strong positive, and outlier. The outlier group consists of dead cells that display high-intensity nuclear staining (Supplementary Fig. S1). For p53 and p63, we obtained six, and for p27, we obtained four classes representing negatively and positively stained cells with different staining intensities. We determined the cutoff value of a positively stained cell for \(\gamma\text{H2AX}\), p53 (DO-7), p27, and p63 as \(y > -0.62x + 1.3\), wherein \(y = \) margination and \(x = \) intensity mean (Supplementary Fig. S1D). For p53 (7F5), the cutoff value was \(y > -0.62x + 1.4\), and for p21, cutoff was \(y > -1.33x + 1.6\).

**Results**

**Ex vivo cultured prostate tissues retain viability and normal cytoarchitecture**

Prostate tissue was collected from patients undergoing radical prostatectomy or cystectomy (Fig. 1A), sliced, and maintained in culture up to 7 days. To confirm that the ex vivo cultured tissues maintained viability and that overall tissue morphology was preserved in culture environment, tissue sections cultured for 1, 2, and 7 days were stained using standard H&E protocol and were evaluated by experienced pathologists (A.S., L.C.A., A.M.D.M.; Fig. 1B). In addition, all tissue sections included in the study were routinely stained with H&E and were confirmed to be nonmalignant. We determined the expression of established compartment-specific markers to ensure the viability of the three major cell types both in cultured prostate tissues (Fig. 1C) and, as control, in standard paraffin-embedded routine sections of the prostate (Supplementary Fig. S2A). Conjugate controls for each staining are provided in Supplementary Fig. S2B. Expression of p63 and cytokeratins 5 and 14 was detectable in the basal cell compartment, p27 and AR were expressed in the luminal cell compartment, and α-SMA was expressed by stromal cells at comparable levels to standard paraffin-embedded prostate sections, although AR levels somewhat declined after a week (Fig. 1C; Supplementary Fig. S2A).

In adult prostate, the number of replicating cells is low (<1%; ref. 26). Although the culture medium is serum free, it is supplemented with mitogenic factors and androgen analogue R1881 that could support replication. Based on Ki67 expression and 5-BrdUrd incorporation, only a few replicating cells were comparable with standard paraffin-embedded prostate sections in the cultured prostate tissue sections during the first 2 days (Fig. 1D; Supplementary Fig. S2C). The number of Ki67-positive cells increased somewhat after 7 days in culture. Interestingly, 5-BrdUrd–positive cells were found especially in the rims of the tissue sections with glands exposed to sectioning, where epithelial cells seem to be recruited to cover the exposed surfaces (Supplementary Fig. S2C).

As a marker for luminal cell function, we tested for production of prostate-specific antigen (PSA) by the cultured tissues. We collected tissue culture medium at various time points and measured total PSA levels. On average, PSA levels increased to 1,700 and 2,900 \(\mu\text{g}\text{L}^{-1}\) after 24 and 48 hours in culture, respectively (Supplementary Fig. S3A), which are ∼1,000-fold higher than levels present in plasma and 1,000-fold lower than levels measured in the seminal fluid of healthy men (27). There was a steady increase of PSA in the culture medium from 2 to 22 hours (Supplementary Fig. S3B). PSA was also detected in the apical surfaces of the luminal cells in the cultured prostate tissues (Supplementary Fig. S3C). Based on the intact cytoarchitecture, expression of several compartment-specific proteins, and production of an androgen-dependent marker, PSA, we conclude that the ex vivo cultured prostate tissues are a tenable model for the analysis of human tissue level responses to DNA-damaging agents.

**Divergent activation of \(\gamma\text{H2AX}\) and p53 in nonreplicating prostate tissue**

We have earlier shown that prostate epithelial cells have attenuated DNA damage checkpoint and p53 responses (24). We therefore wanted to assess how the normal prostate tissue recognizes different forms of DNA damage. We used \(\gamma\text{H2AX}\) as a rapid marker for DDR and p53 as a more universal marker for cellular stress. We selected incubation times to represent early and late responses of \(\gamma\text{H2AX}\) and p53 based on DDR in LNCaP prostate cancer and U2-OS osteosarcoma cells (Supplementary Fig. S4). We treated the cultured prostate tissues with IR; topoisomerase poisons doxorubicin, etoposide, and camptothecin; and DNA intrastrand crosslinker cisplatin, which are prominent inducers of p53 and \(\gamma\text{H2AX}\) in replicating cancer cells (Supplementary Fig. S4). To increase the robustness of the analysis, we developed a quantitative image analysis application based on \(k\)-means algorithm that allows identification of cells and their signal intensities (Supplementary Fig. S1). In the analyses of \(\gamma\text{H2AX}\), we excluded, as outliers, cells with high and even \(\gamma\text{H2AX}\) staining and which displayed condensed chromatin and fragmented nuclei consistent with cell apoptosis (Supplementary Fig. S1; refs. 28, 29). To ascertain that the outlier cells were in fact apoptotic, we stained the...
tissues for γH2AX and cleaved caspase-3, a known apoptotic marker. This showed colocalization of the two signals confirming that the γH2AX outlier analysis is suitable as an apoptotic marker (Fig. 2D; Supplementary Fig. S5).

At 8 hours, γH2AX foci were robustly induced by IR and to a lesser extent by doxorubicin, etoposide, and camptothecin (Fig. 2A and B). Cisplatin did not affect γH2AX. At 24 hours, the intensity of γH2AX staining by IR, doxorubicin, and...
etoposide was reduced, possibly due to repair of the DNA damage (Fig. 2B; Supplementary Fig. S6). Camptothecin was the strongest and most consistent inducer of p53 in the prostate epithelium at 8 and 24 hours (Fig. 2; Supplementary Fig. S6). Doxorubicin stabilized p53 in some but not in all patient specimens (Fig. 2; Supplementary Fig. S7). IR increased the number of p53-positive cells in some patient specimens, but compared with occasional positive cells in the control, there was no significant increase in the p53 intensity (Fig. 2B and C). Furthermore, there was a lack of correlation in the induction of p53 and expression of γH2AX foci by the different treatments. This was evident especially following IR treatment where in abundantly γH2AX-positive sections only a few cells stained weakly for p53 (Fig. 2A and B; data not shown). Conversely, camptothecin, which most strongly induced p53 at 8 hours, did not elicit a pronounced γH2AX formation. None of the treatments induced a robust apoptotic response at 24 hours, despite the relatively high drug and IR doses used (Fig. 2D). Furthermore, at single cell level, there was no correlation between p53 expression and the apoptotic response (data not shown).

Figure 2. Divergent γH2AX and p53 responses to cytotoxic drugs and IR in human prostate tissues. A, tissue slices were treated with doxorubicin (Doxo, 2 μmol/L), etoposide (Eto, 40 μmol/L), camptothecin (Cpt, 2 μmol/L), and cisplatin (Cis, 40 μmol/L) or exposed to IR (10 Gy) and incubated for 8 h, after which the slices were fixed and stained for p53 and γH2AX. Nuclei were counterstained with Hoechst 33342 (DNA). Confocal images of representative fields were captured at 40× magnification. Scale bar, 50 μm. Insets from the indicated regions are enlarged by 3.5-fold. B, quantitative image analysis for p53 and γH2AX. Images were acquired from two to six fields of each specimen (n = 3–4 patients per treatment) and quantified using CISC imaging application. The total number of cells analyzed for each treatment varied from 1,300 to 3,900 cells. Error bars, SD. P values were calculated using Student’s two-tailed t test (*, P < 0.05; **, P < 0.01; ***, P < 0.001) compared with control. C, relative intensity of p53 expression. The mean intensity of p53-positive nuclei, analyzed as in B, was normalized by the mean intensity surrounding each nucleus. P values were calculated using Student’s two-tailed t test (*, P < 0.05; **, P < 0.01; ***, P < 0.001) compared with control. D, tissues were treated as in A, incubated for 8 or 24 h and analyzed for the fraction of γH2AX outliers (left). Immunostaining of control and camptothecin-treated tissues for cleaved caspase-3 (green) and γH2AX (red; right). Nuclei were counterstained with Hoechst 33342 (DNA). Confocal images were captured at 40× magnification. Scale bar, 50 μm. Insets from the indicated regions are enlarged by 2.9-fold.
The apparent sensitivity of the prostate cells to stabilize p53 by doxorubicin and camptothecin is interesting considering that the prostate is a largely nonreplicating tissue and that the topoisomerase poisons mainly act by causing DNA DSBs in replicating cells (30–32). We have earlier shown that p53 is induced in the prostate tissue by nutlin-3, a negative regulator of MDM2, and a nuclear export blocker leptomycin B (24). We hence tested whether inhibition of proteasome function or RNA polymerase I activity, known effectors of p53 pathway, would regulate p53 activity in prostate. As shown in Supplementary Fig. S8, treatment of the prostate tissues with MG132 or actinomycin D resulted in robust p53 accumulation in epithelial cells. These results indicate that the prostate epithelium displays expected responses to transcriptional and proteasomal stress.

To address whether the apparent lack of p53 stabilization leads to a defect in the activation of a known p53 downstream target, we assessed for regulation of p21. Camptothecin was the most prominent inducer of p21 (Fig. 3). The fraction of p21-positive cells correlated quite well with p53 expression ($R^2 = 0.8137$), although the responses varied greatly between the patient specimens (Fig. 3A and C; Supplementary Fig. S7).

**Figure 3.** p21 expression correlates with p53 responses. Tissue slices were treated with doxorubicin (Doxo, 2 μmol/L), etoposide (Eto, 40 μmol/L), camptothecin (Cpt, 2 μmol/L), cisplatin (Cis, 40 μmol/L), and IR (10 Gy) and incubated for 24 h, after which the slices were fixed and stained for p21 and p53. Nuclei were counterstained with Hoechst 33342. Confocal images of representative fields were captured at 40× magnification. Scale bar, 50 μm. Insets, 3.8-fold enlargement. B, quantitative image analysis for p21 using CISC imaging application from three to six fields of each specimen from two to three patients per treatment (1,500–3,300 cells per treatment). Error bars, SD. $P$ values were calculated using Student’s two-tailed t test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) compared with control. C, correlation analysis of p53 and p21 expression in two patient specimens.
Figure 4. p53 is stabilized in basal and luminal cells. A, prostate tissues were incubated with doxorubicin (Doxo, 2 μmol/L) and camptothecin (Cpt, 2 μmol/L) or treated with IR (10 Gy) for 24 h, after which the tissues were fixed and stained for p53 and p63. Nuclei were counterstained with Hoechst 33342 (DNA). Confocal images of representative fields were captured at 40× magnification. Scale bar, 50 μm. Insets, 3.3-fold enlargement. B, p53 and p63 expression was quantified using CISC from three to four fields of each specimen from two patients per treatment (1,400–1,900 cells per treatment). Altogether, 1,800 basal and 4,950 nonbasal cells were analyzed. Error bars, SD. P values (**, P < 0.01) represent comparison of basal and nonbasal cells.
Disparate γH2AX responses to DSB in the prostate epithelial compartments

Epithelial and stromal cells are relatively radioresistant, and in general, rapidly replicating cells are more prone to the effects of cytotoxic drugs. Given the variability in the γH2AX and p53 responses, we analyzed the cell type specific responses by coimmunostaining γH2AX and p53 with basal and luminal markers, p63 and p27. The images were further subjected to quantitative coexpression analyses. Of the three major cell types, p53 response was evident both in basal and luminal epithelial cells (Fig. 4A and B). Stabilization of p53 was not detected in stromal cells by any of the treatments.

Surprisingly, following DSB inducing treatments, like IR, only low levels of γH2AX were detected in luminal cells, although γH2AX foci were clearly visible in the adjacent basal and stromal cells (Fig. 5A and B; Supplementary Fig. S9). At earlier time points (1–4 hours) when γH2AX signal was maximal, we could detect some γH2AX-signal also in the luminal cells, although it was considerably lower than in the basal cells.

Figure 5. Cell type–specific induction of γH2AX. A, tissue slices were treated with IR (10 Gy) for 8 h, after which the slices were fixed and stained for γH2AX and p27. Scale bar, 50 μm. Insets, 2.8-fold enlargement. B, γH2AX and p27 expression was quantified using CISC from two to four fields of each specimen from two patients per treatment (600–1,300 cells per treatment). Error bars, SD. P values (*, P < 0.05; **, P < 0.01; ***, P < 0.001) represent comparison of luminal and nonluminal cells. C, immunohistochemistry staining of H2A.X in paraffin-embedded prostate tissue. Scale bar, 100 μm. Inset, 2.5-fold enlargement. D, H2AX and p27 staining in ex vivo cultured prostate tissue. Scale bar, 50 μm. Insets, 2.8-fold enlargement.
lower than in basal cells and stroma (Supplementary Fig. S9B; data not shown). To investigate this further, we stained standard paraffin-embedded prostate tissue and cultured tissue specimens to clarify whether total H2A.X was expressed in luminal cells. Compared with the level of H2A.X present in the basal cells, the expression of this histone variant in the luminal compartment was negligible (Fig. 5C and D).

**pATM and 53BP1 foci are sustained both in basal and luminal cells**

γH2AX is considered an essential scaffold protein for the retention of DDR proteins on the chromatin at or near DNA DSB (11, 16, 17, 19, 20). Also, given that the lack of H2A.X leads to increased genomic instability, we wanted to assess the extent of activation of the DDR pathway by analyzing pATM and 53BP1 foci formation. IR, doxorubicin, and etoposide induced pATM foci in both luminal and basal cells, whereas camptothecin or cisplatin did not (Fig. 6A; Supplementary Fig. S10). pATM foci colocalized with γH2AX foci in the basal cells and persisted even at 24 hours after IR (Supplementary Fig. S10B).

Pan-nuclear 53BP1 was expressed in most luminal cells of untreated prostate tissue (Fig. 6B; Supplementary Figs. S11 and S12). After IR, doxorubicin, and etoposide treatments,
53BP1 accumulated into damage foci in basal, stromal, and luminal cells (Fig. 6B; Supplementary Fig. S11), and the foci were still detectable by 24 hours (Supplementary Fig. S12; data not shown). Thus, the retention of pATM and 53BP1 in damage foci of the prostate luminal cells seems to require little γH2AX.

**Discussion**

The current concepts of prostate cancer biology are based on nonprimate animal models and few available human prostate cancer cell lines. These are unsatisfactory surrogates for the human disease. We present here an alternative method to assay how different cell types respond to DNA damage in ex vivo cultured human prostate tissues. The method provides a unique means to experimentally test drugs and their combinations in live human normal and tumor tissues and to assess possible interindividual variations. Based on quantitative image analyses for DDR proteins in prostate tissues treated with IR and cytotoxic drugs, we show that the DNA damage stresses activate divergent responses. Notably, despite robust activation of ATM kinase cascade, the luminal cell compartment lacks γH2AX response, and p53 pathway is activated by only a subset of the treatments. These findings indicate that the DNA damage stresses activate a distinct set of responses in a cell compartment–dependent manner.

pATM and 53BP1 foci were detected in IR-, doxorubicin-, and etoposide-treated epithelium and stromal cells indicating that the DDR upstream pathway was activated (Supplementary Table S2). However, γH2AX phosphorylation was nonexistent in the luminal cells in response to these treatments. The finding is surprising as γH2AX foci formation is considered essential for the assembly of the adaptor proteins 53BP1, MDC1, and BRCA1 (33). This raises the question whether other factors besides γH2AX stabilize damage foci onto chromatin. Possible candidates could be H3 and H4 acetylation or methylation required for 53BP1 focus formation (34) or that even early, transient γH2AX response is sufficient to initiate accumulation of the downstream proteins. Additionally, higher doses of IR may bypass the requirement for γH2AX or MDC1 (35, 36). γH2AX phosphorylation was also detected in response to camptothecin treatment, which did not activate ATM. As the prostate represents a nonreplicating tissue, it is plausible that, in addition to apoptotic γH2AX signal, γH2AX activation in postmitotic cells occurs consequent to transcription-mediated lesions and may be activated by other PI3Ks like ATR or DNA-PK (32, 37, 38).

p53 response was launched most prominently in response to camptothecin and doxorubicin and correlated with an increase in p21. The relevance of p21 induction in nonproliferating cells is not evident. Given the lack of apparent apoptotic response to the relatively high DNA-damaging treatments, p21 induction may reflect an antiapoptotic response (39). Doxorubicin displayed patient-to-patient variation in p53 stabilization in the nonpathologic glands, which could depend on the levels of topoisomerase II or other underlying causes resulting in the interindividual variation (31). Nucleolar disruption has been proposed as a common denominator for p53-inducing stresses (40), which could explain why treatments causing transcriptional stress, like actinomycin D, led to prominent p53 stabilization in prostate tissue. Interestingly, our results also indicate that the epithelial cells are more prone than stromal cells to p53 stabilization. It is possible that in stromal cells p53 stabilization is hindered by alternative signals or that p53 is transcriptionally silenced. IR, however, was not a potent stabilizer of p53 in the prostate. Whereas we observe an increase in the fraction of p53-positive epithelial cells from 3% to 9%, there is no significant difference in the intensity of the p53 signal. Lastly, our results indicate that ATM activation alone is not sufficient for acute p53 stabilization in the prostate.

Histone variant H2AX comprises 10% of the total H2A in mammalian cells (11, 15). Unexpectedly, compared with basal and stromal cells, luminal epithelial cells express low levels of H2AX. This difference was observed both in ex vivo cultured and standard paraffin-embedded prostate specimens in histologically normal glands. Consequently, the γH2AX response of the luminal cells was greatly diminished by all DNA-damaging treatments. Following IR, faint foci were observed only with high doses of radiation (10 Gy) at early time points (<4 hours). This indicates that even if a low level of phosphorylation of H2AX is attained, the requisite expansion of the γH2AX signal over large chromatin areas is likely lacking in the luminal cells. Histone variant H2AX isoforms are transcribed in replication-dependent and -independent manners (41), so it is plausible that its expression levels are determined by the differentiated state of the cells. Chromatin compaction has been indicated to affect the extent of DDR and γH2AX foci formation in cultured cells (42–44), further suggesting that the chromatin epigenetic state may determine the extent of DDR in differentiated cells.

The lack or low levels of H2AX predisposes to genomic instability and repair defects in fibroblasts, mouse embryonic cells, and lymphocytes (17, 18, 45, 46). Therefore, a low level of H2AX potentially renders the cells unable to launch a full DDR in the presence of DNA damage. Luminal cells are postmitotic, which would make them less susceptible to tumorigenesis. However, luminal cells are predisposed to inflammatory atrophy associated with increased proliferation (47) and transformation by MYC leading to Nkx3.1 downregulation (48), indicating their vulnerability to cell stress and tumorigenesis. In addition, a luminal stem cell capable of reconstituting luminal compartment was described in mice, and **Pten** deletion led to rapid formation of carcinomas, suggesting that these cells represent a population prone for transformation (49). Further studies are needed to assess whether similar luminal stem cells exist in humans and whether they express H2AX and are capable of proficient repair of DNA DSBs.
Disclosures of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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