Hyperthermia Activates a Subset of Ataxia-Telangiectasia Mutated Effectors Independent of DNA Strand Breaks and Heat Shock Protein 70 Status


Abstract

All cells have intricately coupled sensing and signaling mechanisms that regulate the cellular outcome following exposure to genotoxic agents such as ionizing radiation (IR). In the IR-induced signaling pathway, specific protein events, such as ataxia-telangiectasia mutated protein (ATM) activation and histone H2AX phosphorylation (γ-H2AX), are mechanistically well characterized. How these mechanisms can be altered, especially by clinically relevant agents, is not clear. Here we show that hyperthermia, an effective radiosensitizer, can induce several steps associated with IR signaling in cells. Hyperthermia induces γ-H2AX foci formation similar to foci formed in response to IR exposure, and heat-induced γ-H2AX foci formation is dependent on ATM but independent of heat shock protein 70 expression. Hyperthermia also enhanced ATM kinase activity and increased cellular ATM autophosphorylation. The hyperthermia-induced increase in ATM phosphorylation was independent of Mre11 function. Similar to IR, hyperthermia also induced MDC1 foci formation; however, it did not induce all of the characteristic signals associated with irradiation because formation of 53BP1 and SMC1 foci was not observed in heated cells but occurred in irradiated cells. Additionally, induction of chromosomal DNA strand breaks was observed in IR-exposed but not in heated cells. These results indicate that hyperthermia activates signaling pathways that overlap with those activated by IR-induced DNA damage. Moreover, prior activation of ATM or other components of the IR-induced signaling pathway by heat may interfere with the normal IR-induced signaling required for chromosomal DNA double-strand break repair, thus resulting in increased cellular radiosensitivity. [Cancer Res 2007;67(7):3010–7]

Introduction

Hyperthermia is a potent radiosensitizer currently under clinical investigation as a means to improve the response to ionizing radiation (IR)–based cancer treatments (1). Hyperthermia itself has several cellular effects that should be synergistic with IR-induced tumor cell killing (2, 3). For example, unlike the IR response (4), neither hypoxic nor plateau-phase cells are resistant to heat-induced cell killing (5). Despite the complementary nature of radiation- and hyperthermia-induced cell killing, the precise mechanism(s) of heat-induced cell death is not yet clear, and the synergistic interaction between heat and IR in cell killing is even less well understood. Because phase III clinical trials have shown significant benefits from adding hyperthermia to radiotherapy regimens for a number of malignancies (6), understanding these mechanisms has become clinically important.

The primary cellular effect of IR is production of DNA strand breaks with subsequent activation of cell signaling pathways that result in either DNA repair and survival or apoptotic cell death. Hyperthermia may influence either primary signaling events or downstream events such as those dependent on activation of transcription factors like p53 or nuclear factor κB (NF-κB; refs. 7, 8). For instance, inhibition by heat of IR-induced NF-κB activation is well established (8). Hyperthermia enhances X-ray killing in cells derived from both normal and ataxia-telangiectasia individuals. However, normal cells that were allowed to recover at 37°C during the interval between the heat and X-ray treatments do not exhibit enhanced radiosensitivity whereas ataxia-telangiectasia cells remain sensitive (9). Thus heat, or the heat shock proteins induced by heating, may be modulating ataxia-telangiectasia mutated protein (ATM) function and affecting cell survival. In support of this hypothesis, mouse embryonic fibroblasts lacking heat shock protein (Hsp) 70.1/3 are more sensitive to IR alone or in combination with hyperthermia (10). Similarly, reducing cellular Hsp70 (Hsp72) levels caused human cells to become more radiosensitive (11).

Unstressed cells contain inactive ATM in a dimer or higher-order multimer form. Recent studies suggest that chromatin alterations induce rapid autophosphorylation of ATM at Ser1981, which causes dimer dissociation and initiates cellular ATM kinase activity (12, 13). Heat is known to induce chromatin alterations (14–17); however, whether heat-induced chromatin alterations are similar to those induced by IR exposure and whether the corresponding ATM-regulated signaling pathways are activated is not clear.

Recent studies have shown that heat shock induces γ-H2AX foci formation (18); however, it is not certain whether the heat-induced appearance of γ-H2AX is ATM dependent or whether heat-induced γ-H2AX foci are the consequence of chromosomal DNA double-strand breaks. Because IR is known to induce phosphorylation of H2AX and the inactivation of ATM delays IR-induced H2AX phosphorylation, we tested whether the formation of heat-induced γ-H2AX was ATM dependent. We further determined whether heat treatment has any influence on the formation of IR-induced...
γ-H2AX foci and whether such appearance is Hsp70.1/3 or chromosomal DNA strand break dependent. In the present study, we establish that heat-induced γ-H2AX foci appearance is ATM dependent but Hsp70.1/3 independent. Moreover, heat pretreatment did not abrogate IR-induced γ-H2AX foci appearance in ATM-deficient cells. In addition, heat also induced ATM phosphorylation and MDC1 foci formation but, unlike IR, neither 53BP1 nor SMC1 or ATM foci were induced. Our results argue that heat-induced γ-H2AX foci may not be identical to those induced by IR and suggest that heat induces chromatin alterations, which can modulate sister chromatid exchanges (19) but not chromosome DNA strand breaks. It seems, therefore, that heat can activate a subset of components associated with IR-induced cell signaling.

**Materials and Methods**

**Cells.** Mouse embryonic fibroblasts with and without Hsp70.1/3 (Hsp70.1/3+/+ and Hsp70.1/3−/−), mouse kidney fibroblasts with and without ATM (Atm+/+ and Atm−/−), and 293 cells were maintained following published procedures (10). A-TLD1 cells were maintained as described (20). Heat shock treatments were carried out at 43°C for different time periods as shown in the respective figures.

**Western blot analysis, immunoprecipitation, and ATM kinase assays.** Cell lysate preparations, immunoblotting, and detection of specific proteins were done according to previously described procedures (12, 21). For immunoprecipitation, cells were lysed in lysis buffer and precleared with protein A/G beads. Proteins were immunoprecipitated with specific antibodies and immunoprecipitants were washed with lysis buffer as

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**Figure 1.** Phosphorylation of H2AX following exposure to IR, heat shock, or heat shock combined with IR in cells with and without ATM. A, IR-induced phosphorylation of H2AX in cells with and without ATM. a, mouse fibroblast cells grown on coverslips, irradiated with 1.7 Gy, and fixed at indicated times post-irradiation were examined for γ-H2AX foci by immunofluorescence. b, quantitation of γ-H2AX foci observed at various time points post-irradiation at 1.7 Gy. For each time point, 100 cells were analyzed. Each experiment was repeated thrice and the mean number of foci is plotted against time. c, IR-induced phosphorylation of H2AX as detected by immunoblotting. Exponentially growing cells were irradiated with 2 Gy and incubated for different periods post-irradiation. B, the appearance of γ-H2AX foci after heat shock at 43°C for 30 min. a, immunofluorescence of γ-H2AX foci. b, quantitation of γ-H2AX foci after heat shock. C, quantitation of γ-H2AX foci after heat shock exposure in mixed population of Atm+/+ and Atm−/− cells in the ratio of 1:3, 1:1 and 3:1. D, quantitation of γ-H2AX foci after heat shock followed by IR exposure.
previously described (12, 21). The ATM kinase assay was done using p53 or c-Abi as substrates as described (12, 21).

γ-H2AX immunofluorescence measurements. Cell culture in chamber slides, fixation, and immunostaining were done as previously described (22–24). Fluorescent images of foci were captured with a Zeiss Axioskop 2 mot epifluorescent microscope equipped with a charge-coupled device camera and ISIS software (Metasystems, Altlussheim, Germany). Optical sections through nuclei were captured and the images were obtained by projection of the individual sections as recently described (22). The results shown are from three independent experiments. Cells with bubble-like appearance or micronuclei were not considered for γ-H2AX analysis. Antibodies for MDC1 and SSBP1 were provided by Dr. J. Chen (Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT) and anti–SMC-p957 antibody was obtained from Rockland Immunochemicals Inc. (Gilbertsville, PA).

Chromosomal DNA strand break analysis. Three different assays were used to measure DNA strand breaks after treatment with heat shock, IR exposure, and heat shock + IR exposure. These were (a) pulsed field gel electrophoresis (PFGE), (b) DNA supercoiling changes in nucleoids, and (c) G2 chromosome aberrations (gaps + breaks). In the PFGE, cells treated with heat shock, IR, or both were harvested, embedded in agarose plugs, and lysed in situ. Plugs were washed in Tris-EDTA buffer [10 mmol/L Tris-HCl, 1 mmol/L Na2EDTA (pH 8)] and PFGE was carried out with a CHEF DRIII lysed

In situ. Plugs were washed in Tris-EDTA buffer [10 mmol/L Tris-HCl, 1 mmol/L Na2EDTA (pH 8)] and PFGE was carried out with a CHEF DRIII lysed. The nucleoid assay, DNA supercoiling changes are the consequences of DNA strand breaks and were determined as previously described (26, 27). Briefly, after heat shock or IR exposure, the cells were treated with NaCl and Triton X-100, exposed to increasing concentrations of the fluorescent DNA-intercalating dye propidium iodide, and the diameter of the resulting fluorescent halo of DNA was measured with an image analysis system (26).

Cell-based analysis of individual chromosome damage provides the most sensitive assay for determining the induction of DNA strand breaks after stress. To determine whether heat shock induces chromosomal breaks, cells were treated with the DNA repair inhibitors okadaic acid (0.75 μmol/L; Boehringer Mannheim, Indianapolis, IN) and caffeine (2 mmol/L) 4 h before irradiation. After IR exposure, cells were immediately treated with colcemid. Chromosome aberrations were assessed by counting chromatid breaks and gaps per cell as described (10, 28–31). Two hundred metaphases were analyzed for each point.

Results

Induction of γ-H2AX foci by heat shock is ATM dependent but Hsp70 independent. IR exposure caused γ-H2AX foci formation in both Atm+/+ and Atm−/− mouse cells; however, IR-induced phosphorylation of H2AX is delayed in Atm−/− cells as determined by both foci appearance and Western blot analysis (Fig. 1A). In the absence of ATM, there is a relative delay in both the initial formation and subsequent disappearance of γ-H2AX foci. The kinetics, but not the overall level, of IR-induced γ-H2AX foci formation is therefore dependent on ATM status. Although heat was shown to induce phosphorylation of histone H2AX in mammalian cells (32), it is not known whether ATM is required for heat-induced γ-H2AX foci formation. Mouse Atm+/+ and Atm−/− cells were subjected to a 30-min heat shock at 43°C and then allowed to recover at 37°C for various lengths of time. Immediately following heat treatment, the number of γ-H2AX foci increased in Atm−/− cells to between 34 and 45 per cell, and this number decreased only slightly during the 60-min recovery period at 37°C (Fig. 1B and data not shown). In contrast, there was only a nominal increase in γ-H2AX foci formation in heated Atm−/− cells. Heat, therefore, leads to H2AX phosphorylation with foci formation, and, unlike radiation, ATM is required for both of these events. Mixing experiments (Fig. 1C) with Atm+/+ and Atm−/− cells ruled out the possibility of potential differences in immunofluorescence for heat-induced γ-H2AX foci formation between the two cells lines, further
supporting the argument that the difference in heat-induced γ-H2AX foci appearance is due to ATM status.

To determine whether hyperthermia modulates the appearance of IR-induced γ-H2AX foci in the presence or absence of ATM, the combined effect of heat shock and IR on γ-H2AX foci formation in cells with or without functional ATM was examined. Cells were first heated at 43°C for 30 min, then immediately irradiated with 1.7 Gy. Cells with functional ATM formed more foci per cell after the combined treatment compared with the individual treatments (heat or IR alone; Fig. 1D and data not shown). In fact, the total number of foci was approximately additive at 15 min post-irradiation, when foci appearance was maximum (Fig. 1D and data not shown). Although the appearance of IR-induced γ-H2AX foci in ATM-deficient cells was not influenced by hyperthermia, the appearance of γ-H2AX foci was again delayed in such cells.

One of the major cellular responses to hyperthermia is the up-regulation of Hsp70 levels, a protective response that maintains protein function and inhibits apoptotic cell death under stress conditions. Loss of Hsp70 expression in mouse fibroblasts (Hsp70.1/3−/−) is associated with increased IR sensitivity (10). A cell line deficient for Hsp70.1/3 (10) was used to determine whether Hsp70 expression influences γ-H2AX foci induction. Mouse embryonic fibroblasts (Hsp70.1/3−/+ and Hsp70.1/3−/−) were treated with 2 Gy and the total cellular levels of H2AX and its phosphorylated form (γ-H2AX) were determined. Cells deficient for Hsp70.1/3 showed a minor delay in IR-induced levels of γ-H2AX; however, there was no significant overall change in H2AX levels (Fig. 2A). To confirm our immunoblotting results for IR-induced γ-H2AX, we directly assayed for the appearance of IR-induced γ-H2AX foci in cells with and without Hsp70.1/3 (Fig. 2B). In neither cell type were any significant differences detected in the kinetics of γ-H2AX foci appearance and disappearance or total number of foci. Similarly, in cells treated with heat at 43°C for 30 min, there were no significant differences in the appearance of γ-H2AX foci in cells with and without Hsp70.1/3 (Fig. 2C). Such results suggest that the Hsp70.1 and Hsp70.3 gene products have a minimal role in heat- or IR-induced γ-H2AX foci appearance. Although minor differences in γ-H2AX foci were observed between cells with and without Hsp70.1/3, such differences cannot be attributed to heat shock effects on the cell cycle because both cell lines had similar cell cycle phase distributions post-heating (Fig. 3A). Furthermore, ATM status did not influence heat-induced Hsp70 levels (Fig. 3B), arguing that the induction of Hsp70 following hyperthermia has little involvement in heat-induced γ-H2AX foci appearance.

ATM activation in response to heat. To determine the effects of heat on ATM function, cells were heat shocked for different time periods and then analyzed for ATM autophosphorylation by Western blot analysis. Heat shock induced ATM autophosphorylation in both human and mouse cells (Fig. 4A). In mouse cells, deficiency of Hsp70.1/3 had an insignificant effect on heat- or IR-induced ATM autophosphorylation. When heat treatment was combined with IR, ATM auto-phosphorylation was not affected in any obvious fashion.

We further determined whether heat-induced ATM autophosphorylation is independent of Mre11 status because the Mre11 complex has been shown to facilitate ATM activation on DNA damage (20, 33). Heat shock was able to induce ATM autophosphorylation in A-TLD1 cells, which are deficient for Mre11 function (ref. 33; Fig. 4I). These results suggest that heat-induced ATM autophosphorylation is independent of the Mre11-transduced DNA damage signal. Consistent with the heat-induced autophosphorylation of ATM, heat treatment also enhanced ATM kinase activity as determined by a cell-free kinase assay using either glutathione S-transferase (GST)-p53 or GST-Abl (HP) as substrates (Fig. 4B). To test whether heat treatment could directly activate ATM function, purified ATM was heated at 43°C for 15 min in a cell-free system and an ATM kinase assay was carried out. Heat treatment of purified ATM resulted in the loss of its ability to phosphorylate a

![Figure 3](image)

**Figure 3.** Effect of heat on cell cycle distribution in cells with and without Hsp70.1/3 and heat-induced response of Hsp70.1 in cells with and without ATM. A, the cell cycle distribution after heat shock is identical between cells with and without Hsp70.1/3 as determined by flow cytometric analysis described previously (21, 42). B, heat shock response in mouse cells with and without ATM. Cells were heated at 43°C for 30 min and then examined for Hsp70.1 by Western blotting. **Lane 1,** control; **lane 2,** cells heat shocked and recovered for 1 h; **lane 3,** cells heat shocked and recovered for 2 h.
p53 substrate (Fig. 4C), arguing that heat is not directly enhancing ATM function.

**Influence of heat shock on downstream effectors of ATM signaling.** Several major key components of the genome surveillance network are activated by DNA double-strand breaks. Both 53BP1 and SMC1 become progressively, yet transiently, immobilized on chromatin adjacent to double-strand break within minutes of DNA damage (34). Although heat activates ATM kinase activity and autophosphorylation, the phosphorylated ATM did not form discrete foci (Fig. 5A). To test whether or not heat induces 53BP1, MDC1, and SMC1 foci formation, cells were either heated or treated with 2 Gy and, 30 min later, γ-H2AX, 53BP1, MDC1, and SMC1 foci were detected by immunofluorescence (Fig. 5A and B). Whereas irradiated cells showed γ-H2AX, 53BP1, MDC1, and SMC1 foci formation, heated cells contained only γ-H2AX and MDC1 foci. Thus, heat induces a subset of foci induced following cellular IR exposure.

Heat shock does not induce detectable chromosomal DNA strand breaks. To examine whether heat, like IR, causes direct production of chromosomal DNA strand breaks, three separate techniques were used (Fig. 6). PFGE analysis of cells treated with heat at 43°C for 30 or 60 min did not show any induction of chromosomal DNA strand breaks (Fig. 6A). Heat treatment before radiation exposure also had no significant effect on chromosomal DNA strand break induction by IR (Fig. 6A).

The DNA unwinding-rewinding (halo) assay was also used to determine if heat shock can cause a change in the halo (nucleoid) diameter, an indirect measure of DNA damage (27). The halo diameter was maximum at 7.5 μg/mL concentration of propidium iodide (Fig. 6B). Halo diameter increases due to the unwinding of the DNA supercoils. Using the Halo assay, we observed that heat shock caused a change in the nucleoid halo diameter, but no inhibition of DNA rewinding was observed, which is consistent with an increase in nuclear matrix-DNA anchoring and the absence of DNA strand breaks induced by hyperthermia. IR exposure resulted in a significant inhibition of DNA loop rewrapping, but heat shock actually enhanced DNA loop rewinding. Moreover, the nucleoid diameter (which reflects the length of the DNA loops at maximum relaxation) was reduced post heat shock whereas IR exposure resulted in an increase in nucleoid diameter. Such results are consistent with the previous reports that heat does not induce chromosomal single strand breaks as measured by alkaline elution technique (35).

Finally, we scored the induction of G2-type chromosomal aberrations in heat-shocked cells with inhibited repair of DNA strand breaks. Conditions were first established to measure the chromosome aberrations in cells with and without functional ATM in the presence and absence of repair inhibitors (Fig. 6C). Cells with functional ATM treated with okadaic acid and caffeine had ~4-fold higher chromosome aberrations as compared with untreated cells after IR exposure. The number of chromosome aberrations detected after irradiation with 0.15 Gy is statistically significant as compared with unirradiated cells ($P < 0.001$). Cells deficient in ATM treated with okadaic acid and caffeine displayed a minimum increase in IR-induced chromosome aberrations, suggesting that such treatment in ATM proficient cells resulted in the inhibition of DNA strand break repair. The results showed a striking difference in the ability of ATM+/+ and Atm−/− cells to repair their damaged DNA.

Cells were then exposed to IR doses as low as 0.15 Gy and immediately analyzed for G2-type aberrations. Whereas irradiated
Heat Shock Induces γ-H2AX without DNA Damage

![Figure 5. Influence of heat shock on ATM, 53BP1, SMC1, and MDC1 foci formation and the interaction between γ-H2AX and 53BP1 after heat or IR exposure. A and B, γ-H2AX, 53BP1, SMC1, and MDC1 foci formation in 293 cells 30 min after heat shock (HS; 43°C for 60 min) or IR exposure (1.7 Gy).](image)

cells had detectable levels of chromosomal aberrations after a 0.15-Gy exposure, no aberrations were seen in cells that were treated with heat alone (Fig. 6C). Furthermore, heat shock in combination with IR did not result in increased production of chromosomal aberrations as compared with IR alone. Together these results all support the argument that moderate heat (43°C, 0–60 min) does not induce DNA double-strand breaks. Because heat shock has been shown to enhance S-phase–specific chromosomal aberrations (10), we tested whether γ-H2AX foci induction by heat occurred predominantly in S-phase cells undergoing replication. Interestingly, heat shock induced higher frequency of γ-H2AX foci in S-phase cells as compared with non–S-phase cells (Fig. 6D and data not shown). In contrast, IR exposure resulted in similar frequency of γ-H2AX foci in S-phase as in non–S-phase cells. These results suggest that heat shock induces more chromatin modifications in S-phase cells than the cells in other phases of the cell cycle.

Discussion

Consistent with previous findings, we showed that heat can induce γ-H2AX foci (18, 32). However, heat-induced γ-H2AX foci formation was found to be dependent on ATM. Because ATM is a known DNA damage sensor (36), we attempted to decipher whether the heat-induced γ-H2AX foci are the same as those formed by IR, a DNA-damaging agent. Although heat-induced γ-H2AX foci reflect activation of ATM and its downstream effectors (37–39), the activation of this well-documented pathway may not be the direct result of induced DNA damage. This is evident from the lack of 53BP1 and SMC1 foci formation and the lack of 53BP1 interaction with γ-H2AX after heat treatment (Fig. 5). Takahashi et al. (18) reported that heat-induced γ-H2AX foci appearance, in contrast to the IR-induced γ-H2AX foci response, was linear with heating time but did not correlate with DNA damage as measured by comet assay. Such results are consistent with our previously published results in which heat treatment at 43°C for 30 to 60 min had no effect on G1 or G2 type chromosome aberrations, whereas there was a significant increase in the S-phase–specific chromosomal damage as detected at metaphase (10). This may be the reason that Takahashi and coworkers observed a plateau for DNA damage with increasing heat treatment, indicating a fixed number of potential sites for this chromatin modification, which may coincide with a higher number of DNA replication sites during S phase (18) and increased sister chromatid exchanges post heat shock (19). Wong et al. (40) found that acute heat shock (10–80 min treatment at 45.5°C) induced very few DNA double-strand breaks. However, they observed a preferential increase in double-strand breaks induced in replicating DNA immediately after heating. This increase was hypothesized to be due to a heat-induced increase in the number of single-stranded regions in replicating DNA at or near the replication forks. Consistent with the hypothesis, we observed a higher number of heat-induced γ-H2AX foci in S-phase cells as opposed to non–S-phase cells (Fig. 6D).

Results presented here argue that γ-H2AX foci induced by heat may not be the same as those induced by IR. This assumption is based on the following observations: (a) heat-induced but not IR-induced γ-H2AX foci formation is ATM dependent; (b) heat induces γ-H2AX and MDC1 foci but not 53BP1 or SMC1 foci; (c) heat induces ATM autophosphorylation independent of Mre11 function; (d) heat, in contrast to IR, does not produce chromosomal breaks as determined by three independent assays; and (e) heat treatment induces a relatively higher number of γ-H2AX foci in S-phase than in non–S-phase cells. These observations suggest that heat-induced γ-H2AX foci could be different from those induced by IR exposure, and heat-induced γ-H2AX foci formation does not require chromosomal DNA strand breaks. These results are consistent with the dogma that moderate heat treatment itself does not induce chromosomal DNA strand breaks but can alter chromatin structure as evident from DNA halo (nucleoid) assay (Fig. 6), thus influencing DNA repair and enhancing cellular radiosensitization (41). Although the Hsp70 status did not affect γ-H2AX foci formation following heat treatment or IR exposure,
Hsp70.1/3−/− cells are more radiosensitive compared with Hsp70.1/3+/+ cells (10). Present studies indicate that Hsp70 may function later, downstream of the initial signaling events reflected by foci formation.

Our present study addresses an important issue about whether or not chromatin alterations alone are sufficient to activate ATM, as proposed by Bakkenist and Kastan (13). Our results support the argument that heat can induce chromatin changes as evidenced by

**Figure 6.** Detection of chromosomal DNA strand breaks by various assays after heat shock, IR, and heat shock + IR exposure. A, DNA damage analyzed by PFGE. A, ethidium bromide–stained gel of samples exposed to either IR, heat shock, or heat shock + IR. b, PFGE analysis immediately after exposure to IR, heat shock, or heat shock followed by IR. Columns, mean from two independent experiments; bars, SE. B, DNA strand break measured by Halo assay. a, DNA supercoiling changes in nucleoids (halo) as a measure of DNA strand breaks. b, DNA strand break measurement by analyzing halo (nucleoid) diameter at full supercoiled relaxation in cells after exposure to IR or heat shock and inhibition of rewinding. C, G2 chromosomal aberrations. a and b, G2 chromosome aberration analysis in cells with and without ATM function. Mouse fibroblasts were first treated with okadaic acid and caffeine and then irradiated. The results showed a striking difference in the ability of Atm+/+(a) and Atm−/− (b) cells to repair their damaged chromosomal DNA. c, G2 chromosome aberrations analyzed after exposure to heat shock, IR, or heat shock + IR. For each point, 200 cells were analyzed for chromatid breaks and gaps. Columns, mean from three independent experiments; bars, SE. D, comparison of IR- or heat shock–induced appearance of γ-H2AX foci in S-phase versus non–S-phase cells. Mouse cells were grown on coverslips, labeled with bromodeoxyuridine (BrdU) for 30 min, then irradiated with 1 Gy of γ-rays or treated with heat shock at 43°C for 30 min, and processed for foci detection as previously described (22). Bromodeoxyuridine labeling and γ-H2AX were detected with anti-bromodeoxyuridine and anti-phospho-histone-H2AX antibodies, respectively.
phosphorylation of H2AX and MDC1 foci formation; however, heat itself is not inducing DNA damage because no induction of 53BP1 or SMC1 foci or chromosomal DNA strand breaks are seen. Whereas heat treatment does induce ATM autophosphorylation or enhance its kinase activity, it is possible that heat-activated ATM may not be functionally equivalent to the species induced by IR exposure and thus could impair the signaling pathway associated with DNA damage repair. Such an assumption is consistent with the long-standing observation that heat-induced inhibition of DNA repair is due to an alteration in higher-order chromatin structure (41). Although the detailed mechanism is not yet known, the present studies support a working model for heat-induced chromatin alterations that correlate with activation of ATM in the absence of DNA damage. Further studies are required to determine whether and how heat abrogates ATM sensing to make cells more sensitive to cell killing by IR.

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