Heat Shock Inhibits Radiation-induced Activation of NF-κB via Inhibition of I-κB Kinase*

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Radiation stimulates signaling cascades that result in the activation of several transcription factors that are believed to play a central role in protective response(s) to ionizing radiation (IR). It is also well established that heat shock alters the regulation of signaling cascades and transcription factors and is a potent radiosensitizing agent. To explore the hypothesis that heat disrupts or alters the regulation of signaling factors activated by IR, the effect of heat shock on IR-induced activation of NF-κB was determined. Irradiated HeLa cells demonstrated transient increases in NF-κB DNA binding activity and NF-κB protein nuclear localization. In addition, irradiated cells demonstrated increased I-κB phosphorylation and decreased I-κBα cytoplasmic protein levels, corresponding temporally with the increase of NF-κB DNA binding. Heat shock prior to IR inhibited the increase in NF-κB DNA binding activity, nuclear localization of NF-κB, and the phosphorylation and subsequent degradation of I-κB. I-κB kinase (IKK) immunoprecipitation assays demonstrated an increase in IKK catalytic activity in response to IR that was inhibited by pretreatment with heat. Kinetic experiments determined that heat-induced inhibition of NF-κB activation in response to IR decayed within 5 h after heating. Furthermore, pretreatment with cycloheximide, to block de novo protein synthesis, did not alter heat shock inhibition of IR induction of NF-κB. These experiments demonstrate that heat shock transiently inhibits IR induction of NF-κB DNA binding activity by preventing IKK activation and suggests a mechanism independent of protein synthesis.

More than half of all cancer patients receive radiation therapy, thereby emphasizing the need to understand the cellular and molecular events following exposure to ionizing radiation (IR). IR produces a series of effects on cells, including lethality, cell cycle arrest, and induction of mutations and malignant transformation (1, 2). In addition, IR induces the expression of a variety of cellular genes, termed early response genes. These genes include c-fos, c-jun, egr-1, p53, and nF-κB, which are thought to play a central role in the cellular cytotoxic response to IR (3, 4). These early response genes encode nuclear transcription factors involved in the transmission of intracellular information through multiple signal transduction pathways (5, 6). In this regard, these gene products may function in coupled short-term changes in cellular phenotype by modulating the expression of specific target genes involved in cellular defenses to various stressors, including the effects of ionizing radiation (7, 8). Therefore, IR-induced activation of early genes provides an ideal model system to study the molecular and biochemical events that occur in response to radiation-induced cellular stress.

The cellular stress induced by heat shock or hyperthermia has profound effects on many aspects of cellular biochemistry, morphology, and function and has been shown to greatly enhance the tumoricidal effects of IR (9). This is demonstrated by a reduction in D0 and D50, two parameters that denote the slope and initial shoulder of the clonogenic cell survival curve that represents cell sensitivity to IR (10). As a result of clinical studies over the last 20 years, it appears that there is a significant advantage in the use of heat combined with IR or cytotoxic drugs to enhance tumor cell killing (11). As such, hyperthermic radiosensitization remains a powerful model system to investigate the biochemical and molecular mechanism(s) of radiosensitization. However, despite publication of numerous observations of heat-induced alterations in subcellular structures and signaling systems, no consensus regarding the molecular mechanism of heat-induced radiosensitization has emerged (12). Thus, the specific mechanism(s) of heat-induced alterations in the cellular response to IR remains obscure.

The cellular responses to the elevation of surrounding temperatures are remarkably well conserved across all species from bacteria to mammals and are primarily mediated at the transcriptional level by preexisting transcriptional activators, known as heat shock factors (HSFs) (9, 13–15). In addition to HSFs, it appears that several additional signal transduction cascades are also activated in response to heat including p38/HOG1 kinase (16), Jun N-terminal kinase (17), mitogen-activated protein kinase 1 (18, 19), and protein kinase C (20, 21). It has previously been shown that these signaling pathways are activated by a wide variety of environmental insults, including IR. The cellular parameters that influence the effects of heat shock on the cellular response to IR include molecular oxygen, pH, cell cycle regulation, and DNA repair, all of which rely on signal transduction pathways for the regulation of these processes (10–12). These experiments indicate that the signal transduction pathways activated in the cellular response to elevated temperature may be similar to those elicited by other...
types of environmental stress. These results suggest that coupled or competing interactions between signaling pathways activated by heat shock and IR may be one mechanism responsible for heat-induced alterations in the cellular response to IR.

NF-κB is a dimeric transcription factor activated in response to multiple primary (e.g., viruses, and bacteria) or secondary (e.g., inflammatory cytokines, UV, and IR) agents (22–24). NF-κB is a heterodimer, primarily composed of a 50-kDa DNA-binding subunit (p50) and a 65-kDa transactivator (p65 or rel-A), that is sequestered within the cytosol by association with an inhibitory protein known as I-κB (6, 25, 26). Both the p50 and p65 monomers contain Rel regions, approximately 300 amino acids in length, that bind to DNA, interact with each other, and bind the I-κB inhibitors (25, 27). Activation is post-translational and results from the dissociation of the NF-κB: I-κB complex followed by translocation of the released NF-κB into the nucleus (28, 29). This process leads to increased levels of NF-κB at specific DNA enhancer sequences in the nucleus resulting in the activation of target genes. Phosphorylation targets I-κB for protein ubiquitination and subsequent degradation through a proteasome-dependent pathway, resulting in dissociation of the NF-κB:I-κB complex. In addition, we determined that IR activates the IKK complex resulting in I-κB phosphorylation and degradation of cytoplasmic I-κB resulting in the activation of target genes. Phosphorylation—Electrophoretic Mobility Shift Assays (EMSAs)—EMSAs were performed as described previously utilizing a 32P-radioabeled oligonucleotide containing a consensus NF-κB DNA-binding site (Santa Cruz Biotechnology, Inc.). Briefly, whole cell extracts (10 μg of protein) were isolated as described previously (40) and incubated with poly(dI-dC) for 10 min on ice to bind nonspecific DNA-binding proteins followed by addition of radiolabeled oligonucleotide (approximately 10 fmol, 200,000 cpm of radiolabeled probe/reaction). Samples were incubated at 25 °C for an additional 20 min and 5 μl TBE, glycerol, bromphenol blue solution was added to the samples prior to sample loading. Supershift experiments were performed by adding either anti-p65 or anti-p50 antibody (Santa Cruz Biotechnology, Inc.) to the sample after the initial incubation of the whole cell protein extract-poly(dI-dC) complex. The antibody-protein-poly(dI-dC) complex remained on ice for 10 min, followed by addition of radiolabeled oligonucleotide and incubated for 20 min at 25 °C. For the cold competition experiment, 1 μg of unlabeled NF-κB oligomer was added to the sample after incubation of whole cell protein extract-poly(dI-dC) complex on ice for 10 min. The cold oligomer-protein-poly(dI-dC) complex remained on ice for an additional 10 min, the radiolabeled oligonucleotide was added, and the sample was incubated for 20 min at 25 °C. To inhibit protein synthesis, cycloheximide was added to HeLa cells 45 min prior to heat shock or IR at a final concentration of 10 μg/ml. All samples were run on a 4.5% nondenaturing polyacrylamide gel at 100 V for 45 min. Gels were dried and exposed to a phosphorimager screen for quantitation of incorporated radioactivity. HeLa cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in DMEM containing 250 mM NaCl, 50 mM HEPES (pH 7.4), 1 mM EDTA, and 1% nonessential amino acids (Gibco, Inc.). Western blotting analysis was performed using polyclonal antibodies to NF-κB (p65), I-κBα (Santa Cruz Biotechnology, Inc.), and phosphoserine proteins (Sigma). The nitrocellulose filter was blocked in a 5.0% milk, PBS, 0.01% Tween solution for 1 h followed by the addition of antibodies diluted 1:1000 in 5% nonfat dry milk, PBS, 0.01% Tween solution as per the manufacturer’s specifications and hybridized overnight at room temperature. The nitrocellulose filter was then washed in PBS-Tween for 15 min and incubated with appropriate secondary antibody (anti-goat or anti-rabbit IgG (Fc) conjugated with horseradish peroxidase (1:1000 dilution)) at room temperature for 1 h. The blot was again washed three times in PBS-Tween for 15 min and then developed using an enhanced chemiluminescence method (Amersham Pharmacia Biotech) on x-ray film (Eastman Kodak). Immunoprecipitation Western Analysis—HeLa cells were washed twice with PBS, scraped off the plates in 1 ml of PBS-2.5 mM EDTA, and spun down at 12,000 rpm for 30 s at 4 °C. Cells were lysed in ELB buffer containing 250 mM NaCl, 50 mM HEPES (pH 7.4), 1 mM EDTA, and 1% Nonidet P-40, with 1 mM PMSF, 5 μg/ml aprotinin, and 5 μg/ml leupeptin for 30 min. Lysates were centrifuged at 12,000 rpm for 30 s, and the supernatant was precleared with protein A-agarose, then immunoprecipitated by incubation with the anti-I-κB antibody and protein A at 4 °C for 2 h. The samples were centrifuged for 1 min, and the pellets washed three times in lysis buffer. Pellets were resuspended in 1× running buffer, boiled for 5 min, run on a denaturing SDS-polyacrylamide gel, and transferred to nitrocellulose followed by Western analysis as above.

**Preparation of Cellular Extracts and Nuclear and Cytoplasm Subcellular Fractionation—**Preparations were prepared for analysis from whole cells by a rapid method, modified from Dignam (40). For the isolation of nuclear and cytoplasmic protein extracts HeLa cells were washed twice with phosphate-buffered saline (PBS), scraped off the plates in 1 ml of PBS, 2.5 mM EDTA, and spun down at 14,000 rpm for 2 min at 4 °C. The supernatant was collected by centrifugation at 100,000 g for 15 min on ice. Nonidet P-40 was added to a final concentration of 0.1%, the cellular suspension was vortexed for 10 quick mixings and microcentrifuged at 14,000 rpm for 5 min at 4 °C. The supernatant, representing the cytoplasmatic fraction, was removed and both the supernatant and cell pellet were stored at −80 °C overnight. The nuclear pellet was removed using ice for 15 min and suspended in 100 μl of extraction buffer (10 mM HEPES, pH 7.4, 422 mM NaCl, 2 mM EDTA, 0.1 mM dithiothreitol, and 200 μM PMSF), incubated at 4 °C for 30 min, and spun for 5 min at 14,000 rpm. The supernatant, representing the nuclear fraction, was added to a new Eppendorf tube, and the pellet and supernatant were stored at −80 °C. Protein concentrations were determined using the Bradford method (as per the manufacturer’s specifications).
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**RESULTS**

Heat Shock Inhibits Radiation-induced Activation of NF-κB DNA Binding—The activation of NF-κB following exposure to IR has previously been demonstrated in several cell types (24, 39). To investigate the process in detail, we first determined the exact time course of NF-κB activation and the radiation dose required to elicit this effect in HeLa cells. HeLa cells were exposed to 10 Gy of IR, whole cell extracts were prepared, and NF-κB DNA binding activity was assessed by EMSAs using an oligomer containing a consensus NF-κB site (κB). As shown in Fig. 1A, the DNA binding activity of NF-κB increased roughly 8-fold at 2 and 4 h (lanes 2 and 3) following IR, and this induction returned to baseline levels at 6 h (lane 4). The induction of NF-κB DNA binding was first observed between 45 and 60 min (data not shown). Sequentially lower doses of IR produced less NF-κB-DNA binding in a dose-dependent fashion (data not shown).

We have previously shown that heat alters the DNA binding activity of c-Fos/c-Jun (34), suggesting that, similar to other forms of environmental stress, heat alters the activity of transcription factors. Because heating of tumor cells significantly alters the cellular response to IR (10, 12), we hypothesized heat shock may alter the cellular signaling pathways induced by IR. To examine whether heat shock has any effect upon other nuclear transcription factors, the DNA binding activity of NF-κB was determined following exposure to heat shock. These results indicate no induction or a slight decrease in NF-κB DNA binding following heat shock (Fig. 1B). In contrast to cells exposed to IR alone, treatment with heat prior to IR completely abolished the IR-induced increase of NF-κB DNA binding observed at 2 and 4 h (Fig. 2A, lanes 3 and 5 versus lanes 4 and 6). These results suggest that heat shock interferes with IR-induced signaling pathways regulating NF-κB.

Supershifts of IR-induced Activation of NF-κB with Anti-p65 and p50—The NF-κB complex consists of either a p50:p50 homodimer and/or a p50:p65 heterodimer that are activated...
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**Fig. 3.** The effect of heat, irradiation, or heat shock prior to irradiation on NF-\(\kappa\)B and I-\(\kappa\)B protein levels. Control, heated, or heated prior to IR HeLa cells were harvested to separate the nuclear cellular fraction from the cytoplasmic fraction. A, the nuclear cellular fractions were analyzed for immunoreactive NF-\(\kappa\)B (\(p_\delta\)) protein levels from cells that were heated (top panel), irradiated (middle panel), or heated prior to IR (bottom panel). 10 \(\mu\)g of cellular protein was separated by SDS-polyacrylamide gel electrophoresis, transferred onto nitrocellulose, and processed for immunoblotting with goat polyclonal antibodies to the \(p_\delta\) subunit of NF-\(\kappa\)B (Santa Cruz Biotechnology, Inc.). B, cytoplasmic subcellular fractions were analyzed for I-\(\kappa\)B protein levels from cells that were heated (top panel), irradiated (middle panel), or heated prior to IR (bottom panel). 30 \(\mu\)g of cytoplasmic protein was analyzed as above and immunoblotted with goat polyclonal antibodies to I-\(\kappa\)B (Santa Cruz Biotechnology, Inc.).

Following exposure to multiple agents. To investigate whether IR-induced activation of NF-\(\kappa\)B DNA binding was due to an increase in the DNA binding of \(p_\delta\) and/or \(p_\delta\) subunits, supershift experiments were performed. Whole cell extracts from irradiated cells (Fig. 2B, lane 1) were used as controls. Following the addition of anti-\(p_\delta\) antibody, the \(p_\delta:p_\delta\) heterodimer gel band was reduced in intensity (lane 2), and a supershifted band was present (arrow). Similar samples tested with the anti-\(p_\delta\) antibody (lane 3) demonstrated a decrease in the \(p_\delta:p_\delta\) and \(p_\delta:p_\delta\) bands and the presence of a supershifted band. These results indicate that the induction of NF-\(\kappa\)B in response to IR results from increased \(p_\delta\) and \(p_\delta\) DNA binding.

Heat Shock Inhibits Radiation-induced Nuclear Localization of NF-\(\kappa\)B—Although the induction of NF-\(\kappa\)B DNA binding following irradiation has previously been described (24, 27), the cellular signaling pathways responsible for activation have not been extensively studied. To examine these pathways in detail, HeLa cells were irradiated, and cellular extracts were prepared using a subcellular fractionation method to isolate the nuclear and cytoplasmic cellular proteins. In response to IR, Western analysis showed an increase in NF-\(\kappa\)B nuclear protein (using an anti-\(p_\delta\) antibody, Santa Cruz Biotechnology, Inc.) that was first seen at 0.5 h after radiation, reached a maximum at roughly 1.5 h (Fig. 3A), and returned to baseline at 6 h (data not shown). In contrast, treatment of HeLa cells with heat shock prior to irradiation prevented NF-\(\kappa\)B nuclear localization following exposure to radiation (Fig. 3A). The nuclear extracts used in the control samples for heat, IR, and heat prior to IR in Fig. 3A are identical; however, the ECL exposure time was greatly increased to visualize the p\(\delta\) band in the heat and heat prior to IR Western blots. Western analysis confirmed no change in NF-\(\kappa\)B protein levels in the cytoplasm following exposure to heat shock or heat prior to IR, excluding the possibility that heat shock degrades cytoplasmic NF-\(\kappa\)B (data not shown). To exclude the possibility that NF-\(\kappa\)B was lost in the isolation of the cytoplasmic and/or nuclear cellular fractions, the remaining membrane-DNA pellet was analyzed by immunoblotting with anti-NF-\(\kappa\)B. No difference in NF-\(\kappa\)B protein levels was observed (data not shown). These results suggest that heat shock inhibits signal transduction pathway(s) upstream of NF-\(\kappa\)B translocation into the nucleus.

Heat Shock Inhibits Radiation-induced Degradation of Cytoplasmic I-\(\kappa\)B—Although the induction of NF-\(\kappa\)B is activated by tumor necrosis factor treatment and other agents, identifying IKK\(\alpha\) and IKK\(\beta\) as critical targets for I-\(\kappa\)B phosphorylation was observed. These results suggest a mechanism whereby either heat shock inhibits IR-induced activation of the kinase complex that phosphorylates I-\(\kappa\)B or heating alters I-\(\kappa\)B conformation in a manner that prevents kinase access to the I-\(\kappa\)B phosphorylation site(s).

Heat Shock Inhibits Radiation-induced Activation of the I-\(\kappa\)B Kinase Complex—The IKK complex consisting of IKK\(\alpha\) and IKK\(\beta\) is activated by tumor necrosis factor treatment and other agents, identifying IKK\(\alpha\) and IKK\(\beta\) as critical targets for I-\(\kappa\)B phosphorylation...
phosphorylation and NF-κB activation (29, 32). Because heat shock inhibits IR-induced phosphorylation of I-κB, we hypothesized that IR induces the activation of IKK and that exposure to heat shock may inhibit activation. This was investigated by immunoprecipitation of IKKα followed by kinase assays performed with a truncated GST-I-κB (1–138) substrate (42). HeLa cells treated with IR demonstrated increased IKK phosphorylation of I-κB (8-fold). IKK activation was first observed at 30 min (Fig. 5A) and was no longer active at 2 h (data not shown). No increase in phosphorylation was observed in cells heated prior to IR (Fig. 5A). These experiments suggest that at least one mechanism for IR-induction of NF-κB requires activation of the IKK complex and that heat shock inhibits IR-induced activation of the IKK complex. These results strongly suggest that heat-induced inhibition of NF-κB DNA binding involves alterations in cellular signaling pathways that regulate and/or inhibit cytoplasmic protein kinase complexes such as IKK.

Decay Kinetics of Heat-induced Inhibition of Radiation Induction of NF-κB—To investigate the mechanism whereby heat inhibits the induction of NF-κB by IR, the decay kinetics of this phenomenon were characterized. This was investigated by determining the time interval following exposure to heat during which radiation-induced activation of NF-κB was inhibited (Fig. 5B). Cells that were unheated (lane 1), heated and isolated after 1 h (lane 2), heated and irradiated 1 h after heating (lane 3), and exposed to IR only (lane 4) are shown as controls. Additional HeLa cell samples were either heated and isolated at 4-h intervals (lanes 5 and 7) or heated and then irradiated at 4-h intervals after heating (lanes 6 and 8). As seen, 5 h after heat shock, HeLa cells regained the ability to induce NF-κB DNA binding in response to IR (Fig. 5, lane 5 versus 6), and activation of NF-κB DNA binding was maintained through 9 h (lane 8). These experiments indicate that the inhibition of NF-κB by this particular heat shock is transient (5 h). The transient nature of this process suggests that signaling pathways or factors may directly or indirectly inhibit the induction of NF-κB DNA binding activity.

**Heat Inhibition of NF-κB by IR is Independent of de Novo Protein Synthesis**—The transient inhibition of IR-induced activation of NF-κB by heat shock suggests that post-translational signaling pathways may be involved rather than protein synthetic pathways. To examine this question, cycloheximide was added to HeLa cells 45 min prior to heat shock, IR, or heat shock prior to IR (Fig. 5C). EMSAs of HeLa cells heated prior to IR are shown as a control (lane 1). Cycloheximide was added prior to heat shock (lane 2), prior to IR (lane 3), or prior to heat followed by IR (lane 4). Sections of fluorograms from EMSAs are shown. Arrows indicate the position of NF-κB and nonspecific DNA-binding complexes (NS).

**DISCUSSION**

Since its initial discovery, NF-κB has emerged as a central component of the inducible cellular transcriptional machinery.
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that is essential for a variety of functions, including growth, immunity, and T-cell activation (22). A hallmark of NF-κB is its extraordinary capacity to respond to a diverse range of both physiological and pathological forms of environmental stress, including, but not limited to, ionizing radiation (2, 24, 44, 45). It has been suggested, as a result of work in several different laboratories, that early genes that function as transcription factors may play a role in tumor cell survival following exposure to IR (1, 2). Hence, NF-κB provides an excellent paradigm to study the role of signaling pathways and the regulation of transcription factors in the cellular response to IR-induced stress.

Hyperthermia has a long history as a treatment modality for cancer and has recently been demonstrated to be an excellent adjuvant to radiotherapy. These results were consistent with in vitro experiments demonstrating the generalized phenomenon of heat-induced radiosensitization (10, 12). The underlying biochemical or molecular biological processes by which heat alters the cellular response to IR has not been firmly established; however, several possible mechanisms have been suggested. These include heat inhibition of the repair of IR-induced DNA damage, alterations in cell cycle progression resulting in tumor cells accumulating at a more radiation-sensitive point in cell cycle, and changes in tumor microcirculation and pH, all of which rely on signaling pathways (12, 46, 47). Despite the variety of hypotheses concerning how heat interacts with IR, the exact mechanism(s) responsible for this process remains unclear.

In this study, we characterized the initial biochemical steps of IR-induced activation of NF-κB, and we report that radiation-induced activation of NF-κB is inhibited by prior exposure to heat shock. Using a biochemical cellular fractionation scheme, it was determined that the mechanism of IR-induced activation of NF-κB involves the phosphorylation and degradation of IκB that temporally precedes NF-κB nuclear localization. These results suggest that radiation-induced activation of NF-κB utilizes a transduction pathway similar to that of several other environmental stressors, including interleukins, tumor necrosis factor, and phorbol esters (22, 44). Kinase assays demonstrated that IR activates IKK and heat shock prior to IR appears to inhibit IKK activation. Inhibition of IKK prevents phosphorylation and degradation of IκB and the subsequent induction of NF-κB DNA binding. The results of these experiments suggest that heat shock, either directly or indirectly, inhibits or alters the cytoplasmic signaling pathway(s) regulating NF-κB.

Heat shock inhibition of IR-induced activation of NF-κB appears to be a transient response and decays by 5 h following heating. This 5-h interval also corresponds to the time period after heating that is required for HSF to return to an inactive DNA-binding state (data not shown). These results suggest a possible temporal relationship between the activation of the heat shock signaling and the inhibition of NF-κB induction by IR, both of which last between 4 and 6 h. The transient nature of heat shock inhibition also suggests a role for intracellular signaling that, in general, is transient. Interestingly, this time frame also closely corresponds to the duration for the decay of heat-induced radiosensitization (13). Finally, cycloheximide was used to demonstrate that heat shock inhibition of IR-induction of NF-κB is independent of de novo protein synthesis, suggesting a mechanism involving cellular signaling pathways. This work supports a growing body of scientific evidence that the activation of signaling pathways by heat shock is involved in more than the cellular response to elevated temperature. Hence, these results raise the possibility that there are coupled or competing interactions between signaling pathways activated by heat shock and IR.

These experiments also identify the IKK complex or other signaling factors upstream of IKK as targets for heat shock inhibition of IR-induced signaling pathways leading to the activation of NF-κB. These results raise the following intriguing questions: 1) how is stress sensed by cellular biomolecules and integrated into pathways that activate NF-κB; and 2) where is the common point at which the competing interactions between heat shock and IR converge? One possibility involves the potential role of the IKKα/β complex as a common and central target molecule (29, 31). Hence, heat shock may inhibit upstream signaling factor(s) that directly activate IKKα/β, perhaps via changes in protein conformation that prevent phosphorylation by upstream kinase(s). Alternatively, heat shock may activate distinct signaling factor(s)/pathway(s) that via competing and/or coupled interactions with IR signaling prevent activation of IKK.

It has been suggested that the induction of NF-κB in tumor cells serves as a reparative or protective mechanism following exposure to agents that induce oxidative stress, such as IR. If this is true, then one possible mechanism of hyperthermic radiosensitization of tumor cells may involve heat inhibition of IR-induced activation NF-κB. This hypothesis fits well with the growing idea that transcription factors play a central role in the cellular response to IR and that in selected tumors, early gene overexpression predicts for clinical outcome with definitive radiation therapy (39, 48). Two lines of previous work suggest a role for NF-κB in cell survival. First, interleukin-3 and the oncogenic TEL/platelet-derived growth factor receptor β fusion protein appear to prevent cell death via activation of NF-κB after cytokine deprivation or exposure to platelet-derived growth factor receptor β inhibitors (48). Second, ataxia telangiectasia cells, which are exquisitely sensitive to irradiation death, become markedly more resistant to radiation death by insertion of an IκBα gene that restores IR-induced activation of NF-κB (27). This result is particularly interesting because ataxia telangiectasia cells have a deficiency in DNA repair that appears to be the major cause of IR-induced cell death (39), and heat-induced radiosensitization appears to result from heat shock inhibition of DNA-repair (10, 12).

Our work suggests that heat-induced inhibition of NF-κB may provide a unique paradigm to delineate a novel mechanism for heat-induced alterations in the cellular response to IR, and thus, expand the knowledge of a fundamental clinical observation, radiosensitization of tumor cells by hyperthermia. Finally, a clearer understanding of heat-induced changes in radiation-induced activation of early response genes and their effects on heat-induced radiosensitization may provide a model system to explore other chemicals or drugs that may have similar effects on early response genes in the process of radiosensitization.

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