Inhibition of Hsp90: A Multitarget Approach to Radiosensitization

Kevin Camphausen1 and Philip J. Tofilon2

Abstract

Hsp90, the 90 kDa heat shock protein, is a highly expressed molecular chaperone that modulates the stability and/or transport of a diverse set of critical cellular regulatory proteins. Among Hsp90 clients are a number of proteins, which in a cell type–dependent manner, contribute to tumor cell radioresistance. Exposure of a variety of solid tumor cell lines to clinically relevant Hsp90 inhibitors results in the simultaneous loss of these radioresponse-associated proteins, which is accompanied by an increase in radiosensitivity. This radiosensitization has been linked to a compromise in the DNA damage response to radiation including the inhibition of cell cycle checkpoint activation and DNA double-strand break repair. With respect to potential clinical application, the expression of ErbB3 seems to predict tumor cells that are resistant to the effects of Hsp90 inhibition on radiosensitivity. Moreover, whereas an increase in tumor cell radiosensitivity was consistently reported, the radiosensitivity of normal fibroblasts was not affected by Hsp90 inhibition, suggesting the potential for tumor-selective radiosensitization. This review summarizes the preclinical data available on Hsp90 inhibition and cellular radiosensitivity. Results generated to date suggest that Hsp90 inhibition can provide a multitarget approach to tumor radiosensitization.

Background

Attempts to develop clinically relevant radiosensitizers have traditionally employed an empirical approach combining radiation with standard cytotoxic chemotherapeutic agents. Although often effective in experimental models, the results obtained when these combinations are applied in a clinical setting have been generally less than expected, primarily due to concomitant increases in radiation-induced normal tissue injury. Based on an increased understanding of the molecular mechanisms of radioresponse, current efforts to develop strategies for enhancing tumor radiosensitivity have focused on the use of agents that target a given molecule putatively involved in regulating radiation-induced cell death. However, complicating this approach, it has also become increasingly clear that cellular radiosensitivity reflects the end result of a combinatorial process comprised of a wide variety of signaling and effector molecules and that the ability of a single molecule to affect radioresponse often depends on the genetic and epigenetic background. Accordingly, there are numerous examples in which targeting a selected radioresponse-associated molecule affects radiosensitivity in a cell type–dependent manner. For example, inhibition of epidermal growth factor receptor, Raf-1, p53, or nuclear factorκB has been shown to enhance the radiosensitivity of some but not all tumor cells (1–8). Given that the prowess of a single molecule to regulate cellular radioresponse is dependent on a variety of genetic/epigenetic circumstances, it would seem that the effectiveness of target-based radiation sensitizers against solid neoplasms will be significantly constrained by intertumor and intratumor heterogeneity.

One approach to overcoming such limitations involves identifying markers that indicate which tumors would be susceptible to a given target-based radiosensitizer. This will require an understanding of the cellular context that allows the respective determinant of radiosensitivity to be operative. In addition to predicting susceptibility as a means of reducing the consequences of cell type specificity, targeting more than one of the potential molecular determinants of radiosensitivity has been suggested as a strategy for increasing the probability and/or degree of radiosensitization. Towards the development of such a multitarget approach to radiosensitization, the molecular chaperone Hsp90 has been the focus of a number of investigations.

Hsp90 and Tumor Cell Radiosensitivity

Hsp90, the 90 kDa heat shock protein, modulates the degradation, folding, and/or transport of a diverse set of critical cellular regulatory proteins (9). Most Hsp90 clients, i.e., those proteins that require its “chaperoning” activity for appropriate function, participate in some aspect of signal transduction including a wide variety of protein kinases, hormone receptors, and transcription factors (10). Hsp90 can also stabilize mutated proteins allowing them to maintain normal function despite genetic abnormalities (11). Many Hsp90 clients are critical oncogenic proteins including ErbB2, Raf-1, Akt, Bcr-Abl, mutant p53, and hTERT (12), which has
generated considerable interest in Hsp90 as a target for cancer treatment. Regarding its potential as a target for radiosensitization, a number of Hsp90 client proteins have been associated with radioresponse (13–17), albeit in a cell type–dependent manner. Thus, whereas most efforts to develop target-based radiosensitizers have focused on individual radioresponse-associated molecules, inhibition of Hsp90 provides an approach for the simultaneous targeting of multiple proteins that can possibly serve as determinants of radiosensitivity.

Investigations into Hsp90 as a potential determinant of tumor cell radiosensitivity have involved the used of the Hsp90 inhibitors geldanamycin and radicicol (natural products) and the clinically relevant geldanamycin analogues 17AAG and 17DMAG. Russell et al. showed that nanomolar concentrations of 17AAG enhanced the radiosensitivity of two glioma and two prostate carcinoma cell lines with minimal cytotoxicity (14). Another clinically relevant Hsp90 inhibitor, 17DMAG, enhanced the radiosensitivity of a glioma and a lung squamous carcinoma cell lines (16). Further investigations of the AsPC1 cell line may provide mechanistic insight into the role of Hsp90 in regulating radiosensitivity. In contrast to the tumor cell lines in which Hsp90 inhibition enhanced their radiosensitivity, ASPC1 cells expressed significant levels of ErbB3. Subsequent studies revealed that other tumor cell lines that expressed ErbB3 were also resistant to 17DMAG-induced radiosensitization (18).

A causal role for ErbB3 in this resistance was established using small interfering RNA to knock down ErbB3 in the resistant AsPC1 cells (18). Whereas individual treatments with small interfering RNA to ErbB3 or 17DMAG had no effect on radiosensitivity, the combination, which reduced both ErbB2 and ErbB3, resulted in a significant enhancement in AsPC1 radiosensitivity. Further investigation focused on the role of ErbB2 and ErbB3 in maintaining the activity of ErbB1, which can provide protection against radiation-induced cell death (22). These studies showed that, although 17DMAG decreased ErbB1 kinase activity in the sensitive MiaPaCa cells through the loss of ErbB2 and the ErbB1/ErbB2 heterodimer, ErbB1 kinase activity was maintained in 17DMAG-treated AsPC1 cells because of the presence of the ErbB1/ErbB3 heterodimer (Fig. 1). Thus, these data suggest that in cells which contain only the ErbB1 and ErbB2 (i.e., cells that contain one type of heterodimer), the loss of ErbB2 after inhibition of Hsp90 was sufficient to reduce ErbB1 activity resulting in radiosensitization. However, in resistant cells, ErbB3 stabilizes ErbB1 activity in the presence of 17DMAG-mediated ErbB2 degradation, which results in no change in radiosensitivity. These data then suggest that ErbB3 expression predicts for tumor cell susceptibility to radiosensitization induced by Hsp90 inhibition.

**Mechanisms of Radiosensitization**

The ErbB3 knock-down studies suggested that the loss of ErbB2 protein and consequent reduction in ErbB1 kinase activity plays a causative role in 17DMAG-induced radiosensitization. However, in subsequent studies, the selective reduction in ErbB2 protein by small interfering RNA and the accompanying reduction in ErbB1 activity had no effect on the radiosensitivity of a panel of non–ErbB3-expressing tumor cells—cells in which 17DMAG induced radiosensitization (18). Thus, whereas the loss of ErbB1 signaling may be necessary for 17DMAG-induced radiosensitization, it is not sufficient, indicating the existence of additional critical targets. Although complicating mechanistic studies, the failure of a single protein to account for the effects of 17DMAG was actually consistent with the rationale for the multitarget approach to tumor cell radiosensitization mediated by Hsp90 inhibitors.

As an experimental approach to identifying the critical target(s), we focused on the effects of Hsp90 inhibitors on the cellular processes that actually mediate radiation-induced cell death. Cellular radiosensitivity is determined by a number of fundamental processes such as apoptosis, cell cycle phase distribution, cell cycle checkpoint activation, and the repair of DNA damage, specifically double-strand breaks (DSB). Delin-eating which of these processes participates in Hsp90 inhibitor–induced radiosensitization should not only provide mechanistic insight, but provide a framework for investigations into the specific molecules involved, i.e., the critical targets. We had initially reported that the radiosensitization induced by Hsp90 inhibition does not involve the synchronization of cells into a radiosensitive phase of the cell cycle or an increase in

**Resistance to Hsp90 Inhibitor-Induced Radiosensitization**

Whereas a number of radioresponse-associated proteins were degraded in response to 17DMAG and 17AAG exposure, radiosensitization seemed to correlate best with a decrease in the levels of ErbB2 (14, 17). This correlation was based on initial evaluations of six tumor cell lines evaluated in vitro or as xenograft tumors are predictive of efficacy in phase II clinical trials (21). It may be possible to extrapolate this analysis to the development of clinically effective radiation modifiers. The Hsp90 inhibitors evaluated to date enhance the in vitro radiosensitivities of cell lines initiated from a variety of human tumors originating from at least five different histologies. Thus, the ability of Hsp90 inhibition to enhance radiosensitivity across a spectrum of human tumor cell lines in vitro suggests that this chaperone protein can serve as a general target for radiosensitization.

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susceptibility to apoptotic death (14, 17). However, the survival of cells after irradiation is heavily dependent on a series of integrated signaling pathways that participate in the repair of DNA DSBs and cell cycle checkpoint activation, which are the principle components of the DNA damage response to ionizing radiation. Moreover, considerable information has been generated regarding the proteins involved as well as their interactions.

Exposure of MiaPaCa pancreatic tumor cells to 17DMAG following a protocol that induces radiosensitization inhibited the repair of DSBs according to gH2AX foci dispersal and the neutral comet assay (15), which reflect two different manifestations of DSB repair. Using this observation to direct subsequent experiments, Dote et al. (15) focused on DNA-PK, which is a critical component of the nonhomologous end joining process. Whereas no effects were detected on the DNA-PK components Ku70 or Ku86, 17DMAG treatment significantly attenuated the radiation-induced phosphorylation (i.e., activation) of the catalytic subunit DNA-PKcs. Of interest, it had recently been shown that DNA-PKcs can be regulated by a direct interaction with nuclear ErbB1, which is transported into the nucleus after irradiation (23); the activity of which is inhibited by 17DMAG (15). Consistent with these previous results, 17DMAG was found to significantly diminish the radiation-induced interaction between ErbB1 and DNA-PKcs. Thus, investigations of Hsp90 have been able to connect a cytoplasmic signaling molecule with a nuclear event that is of consequence in radiosensitivity.

However, these studies do not provide the entire mechanism accounting for Hsp90 inhibitor–induced radiosensitization in that the loss of ErbB1 activity, although necessary, is not sufficient (18). To pursue the hypothesized additional critical targets, studies were extended to another aspect of the DNA damage response—cell cycle checkpoint activation. In these studies, a radiosensitizing exposure to 17DMAG was found to abrogate the activation of the G2 and S phase cell cycle checkpoints (14, 15). Associated with these events was a reduction in radiation-induced ATM activation and foci formation in 17DMAG-treated cells. Although no interaction between ATM and Hsp90 was detected, Hsp90 was found to interact with the MRE11/Rad50/NBS1 (MRN) complex. 17DMAG exposure reduced the ability of the MRN components to form nuclear foci after irradiation. Moreover, 17DMAG exposure reduced the interaction between NBS1 and ATM, although no degradation of the MRN complex was detected. These results suggest that the diminished radiation-induced activation of ATM in 17DMAG-treated cells was the result of a compromise in the function of the MRN complex.

Although the 17DMAG-mediated inhibition of Hsp90 attenuates both the repair of DSBs and the activation of cell cycle checkpoints, whether both effects are necessary and/or sufficient for 17DMAG-induced radiosensitization remains to be determined. The ability of the abrogation of cell cycle checkpoints alone to enhance radiosensitivity has been questioned based on genetic experiments (24, 25) and the inconsistent radiosensitization induced by pharmacologic inhibitors of G2 arrest (2, 26). In contrast, DSB repair is an established determinant of radiosensitivity; inhibition of critical components...
such as DNA-PK genetically or pharmacologically consistently induces radiosensitization (27). However, the effects of 17DMAG on DNA-PKcs activation, which seems to be a consequence of a reduction in ErbB1 activity, was not a complete abrogation, merely a reduction in degree suggesting that Hsp90 (and thus ErbB1) is not an absolute requirement but acts to facilitate DNA-PKcs activity, consistent with the inability of a reduction in ErbB1 activity alone using small interfering RNA to affect radiosensitivity (18).

Most studies related to cancer treatment have focused on cytoplasmic Hsp90 and its role in the stabilization of various kinases. Indeed, the 17DMAG-mediated inhibition of DNA-PKcs activation seems to be the result of a reduction in ErbB1 activity. However, Hsp90 is also found in the nucleus, albeit at considerably smaller levels (28–30). The function of nuclear Hsp90 has not been clearly defined, although it has been shown to participate in the translocation into and retention of steroid receptors in the nucleus as well as the regulation of gene transcription (31–33). The data presented by Dote et al. (15) indicates that nuclear Hsp90 is also bound to the MRN complex. It should be noted that because of the vastly greater levels of Hsp90 in the cytoplasm, similar experiments using whole cell extracts do not yield detectable interactions between these proteins. Thus, it would seem that the nuclear functions of Hsp90 need to be taken into account in investigations and clinical application of Hsp90 inhibitors.

### Tumor versus Normal Cells

Clearly, the therapeutic potential of Hsp90 inhibitors as a radiation modifier will ultimately depend on a selective increase in the radiosensitivity of tumor cells over normal cells. To begin to address this potential, the effects of 17AAG on the radiosensitivity of the nonimmortalized, normal human diploid fibroblast cell line C29-A were evaluated (17). The treatment protocol was the same as that used for the tumor cell lines. However, treatment of C29-A cells with 17AAG had no effect on radiation-induced cell killing. Bish et al. (13) and Noguchi et al. (16) published confirming results showing that 17AAG has no effect on the radiosensitivities of other normal human fibroblast cell lines. 17DMAG was also found to have no effect on the radiosensitivity of the normal human fibroblast cell lines MRC5 and MRC9. Thus, these results indicate that in contrast to a variety of tumor cell lines, Hsp90 is not a determinant of the radiosensitivity of normal fibroblasts. Whether the lack of fibroblast radiosensitization in vitro is predictive of a lack of an enhancement in radiation-induced normal tissue toxicity certainly remains a critical question and is subject to further investigation.

The mechanism responsible for this selective sensitization of tumor cells over normal cells, however, remains unclear. Previous studies have suggested a difference in the biochemistry of Hsp90 in tumor versus normal cells with the chaperone in tumor cells being substantially more susceptible to inhibitors (34). However, this does not seem to be the case with respect to normal fibroblasts and radiosponse-associated proteins. Hsp90 inhibitors reduced the levels of the radiosensitivity-associated proteins Raf, Akt, and ErbB2 in normal fibroblast cell lines (17) in a manner similar to tumor cells and yet, in the fibroblasts, these decreases were not accompanied by an increase in radiosensitivity. Moreover, each of the fibroblast cell lines (C29, MRC5, and MRC9) do not express detectable levels of ErbB3 suggesting that the resistance mechanism identified for tumor cells is not operative in these normal cells. Thus, because inhibition of Hsp90 reduces the levels of the radiosponse-associated proteins in both fibroblasts and tumor cells, affecting only the radiosensitivity of tumor cells, suggests that it is not Hsp90 biochemistry that differs between normal and tumor cells, but the mechanisms through which solid tumor cell lines and normal fibroblasts respond to radiation. Although not explaining the inability of Hsp90 inhibitors to enhance fibroblast radiosensitivity, these results do support the existence of tumor-specific targets for radiosensitization.

### Clinical Application of Hsp90 Inhibitor/Radiotherapy Combination

As a class of drugs, Hsp90 inhibitors enhance radiosensitivity in preclinical models of numerous tumor histologies including but not limited to gliomas and cervical, prostate, and pancreatic carcinomas. Furthest along in clinical development are the geldanamycin analogues 17AAG and 17DMAG. At present, there are 15 open cancer trials of 17AAG including nine phase I and six phase II trials, with eight of those trials using 17AAG as a single agent, and seven of those trials using 17AAG in combination with standard chemotherapy. Because 17DMAG was developed after 17AAG, there are fewer ongoing trials with four active phase I trials. Likewise, there are three phase I studies of CNF2024 (a synthetic, orally bioavailable HSP90 inhibitor), one phase I study of SNX-5422 (a synthetic, novel, small molecule Hsp90 inhibitor), and two phase I studies of IPI-504 (a water-soluble 17AAG analogue; ref. 35), all six of these trials are single agent trials. Although there are no trials currently open for combination therapy of an HSP90 inhibitor and radiation, several are in the approval process.

An important feature of modern clinical trials is the integration of biomarkers as translational end points of drug efficacy. For Hsp90 inhibitors, translational end points are being evaluated as markers of drug effect in several ongoing trials with emphasis on detecting increases in Hsp70 and/or the decrease in Raf-1 or cyclin-dependent kinase 4 (36, 37). With respect to the potential design of clinical protocols combining an Hsp90 inhibitor and radiotherapy, the preclinical results suggest that ErbB3 expression may be useful in patient selection. That is, patients with tumors that do not express ErbB3 would be predicted to respond best to this combined modality. Moreover, data from experimental models indicate that a mode of resistance to Hsp90 inhibitor–induced tumor cell radiosensitization is via the maintenance of ErbB signaling (18). Thus, whereas laboratory studies suggest that ErbB3 expression may be of use in selecting patients, they also suggest that the degree and/or probability of Hsp90 inhibitor–induced radiosensitization may be enhanced if the combined modality included an epidermal growth factor receptor (ErbB1) inhibitor. Clearly, such a combination awaits the initial clinical trials combining Hsp90 inhibitors with radiotherapy.

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3 Unpublished data.

4 http://www.cancer.gov/

5 Personal communication.
References

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