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Inhibiting induction of heat shock proteins as a strategy to enhance cancer therapy

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Abstract
Cancer treatments that incorporate thermal therapy and some systemic therapies induce the production of heat shock or stress proteins. The induced heat shock proteins could lessen the effect of the therapy by inhibiting apoptotic signaling and by acting as molecular chaperones to prevent irreversible cellular damage. Strategies that prevent the induction of heat shock proteins would result in more apoptosis and necrosis, improving the cancer therapy. This paper briefly reviews cancer therapies that induce the stress response, and proposes strategies to reduce the stress response.

Keywords: *Heat shock proteins, apoptosis, acute acidification, quercetin, cancer therapy*

Hypothesis

Thermal therapy induces damage to protein and membrane structures in cells leading to cell death [1–3] (Figure 1). Cells undergo apoptotic and/or non-apoptotic death [4] following thermal therapy and the mode of death is dose and cell type-specific. Thermal therapy also induces the stress response, characterized by induction of heat shock proteins (HSPs). HSPs are molecular chaperones that prevent irreversible inactivation of proteins and target denatured proteins for proteolysis [5, 6]. HSPs also are a class of inhibitor of apoptosis proteins (IAPs) that block apoptotic signalling, inhibit apoptosis and enhance survival [6–9]. Strategies that inhibit the stress response induced by thermal therapy would, therefore, enhance cytotoxicity resulting from both modes of cell death. Inhibition of the stress response would enable apoptosis and/or enhance necrosis. The strategy of inhibiting the stress response is applicable to any modality (e.g. thermal therapy, chemotherapy, radiation therapy) that kills tumour cells through induction of apoptosis as well as necrosis.

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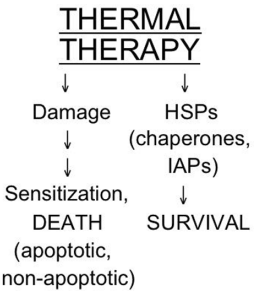

Figure 1. This diagram illustrates that thermal therapy induces damage to various cellular structures and induces the production of heat shock proteins (HSPs). The cellular damage can sensitize the cell to other modalities and result in apoptotic and/or non-apoptotic death, depending on the dose and cell type. HSPs have a dual function. They interact with affected proteins to prevent their irreparable damage and allow for cell survival. HSPs also act as inhibitor of apoptosis proteins (IAPs) to block apoptotic signalling and inhibit proteolysis of critical targets, leading to cell survival. Therefore, preventing the induction of HSPs will (i) prevent repair of damaged cell structures and (ii) enable apoptotic signalling and apoptosis. The end result is enhanced cell death.

Inhibition of the stress response by acute intracellular acidification

Human melanoma cells cultured at acidic pH, a characteristic of regions of solid tumours that may affect treatment outcome [10–14], are resistant to thermal therapy compared to cells cultured at pH 7.3 [15–17]. This is due in part to elevated endogenous levels of HSPs [15, 17]. Acute extracellular acidification inhibits the 42°C-induced stress response and sensitizes the melanoma cells to 42°C [15, 17]. The accompanying reduction of intracellular pH rather than extracellular pH appears to be the critical factor for hyperthermia sensitization [16, 17].

Intracellular pH thresholds were found to exist for melanoma cells growing at pH 7.3 below which the stress response was inhibited and cells were sensitized to 42°C. In contrast, intracellular pH thresholds for heat sensitization did not exist for cells growing at pH 6.7: any reduction in intracellular pH prior to heating resulted in increased cell killing [17]. Since cells grown at low pH lack an intracellular pH threshold for heat sensitization, they are sensitized more to 42°C per unit decrease in intracellular pH than cells grown at pH 7.3, the extracellular pH characteristic of most normal tissues. A reduction of intracellular pH to 6.5 or lower was required to sensitize DB-1 melanoma cells cultured at pH 6.7 to thermal therapy [16]. This is easy to accomplish *in vitro* by changing the pH of the growth medium. It is not as easy to acidify tumours.

The extracellular pH of human tumours subjected to hyperglycemia can be transiently reduced by an average of 0.2 pH unit, while the extracellular pH of normal tissues remain unchanged [18–22]. However, this degree of acidification is not enough to sensitize human tumour cells to hyperthermia [15–17]. Additional strategies need to be employed to enhance tumour acidification for sensitization to hyperthermia. Melanoma cells rely on monocarboxylate transporters (H+ lactate symporters) to remove hydrogen ions under acidic extracellular conditions [23]. A combination of mild acute acidification with an inhibitor of MCTs reduced intracellular pH enough to selectively sensitize melanoma cells cultured at pH 6.7 to hyperthermia by lowering their intracellular pH below the critical threshold value by a treatment that does not lower the intracellular pH below the critical threshold of cells growing at pH 7.3 [16]. It has also been shown that extracellular pH of tumours can be decreased below pH 6.3 by inhibition of MCTs and/or inhibition

of mitochondrial respiration by site 1 respiration inhibitors during hyperglycemia [24, 25]. These findings support the concept for sensitization of human melanoma cells existing in a chronic acidic environment to hyperthermia by strategies that selectively and acutely lower the intracellular pH in acidotic regions of tumours.

HSPs inhibit apoptosis

The proposed strategy of inhibition of the stress response is applicable not only to thermal therapy but also to other modalities used for treating cancer, especially those that induce apoptosis. The heat shock proteins HSP70, HSP27 and HSP90 have been shown to inhibit apoptotic signalling and reduce apoptosis in different model systems [26–29]. Numerous excellent reviews, including those cited previously [6–9], discuss the importance of HSPs as modulators of apoptosis. Therefore, treatment strategies that reduce HSP levels and prevent the induction of HSPs by a therapy should enhance tumour response.

Phase I and II trials are underway with Hsp90 inhibitors, especially the geldanamycin analogue 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) [30–33]. Disruption of Hsp90 function leads to dissociation and loss of function of many of this chaperone's 'oncogenic client proteins' crucial for tumour cell survival [34]. However, this class of benzoquinone ansamycin antibiotics also induces the stress response [35, 36]. The same is true of other Hsp90 inhibitors including the macrocyclic anti fungal antibiotic radicicol [37]. The farnasyltransferase inhibitor manumycin being considered for treatment of mesothelioma and ovarian cancer [38, 39] also induces the stress response [39, 40]. Therefore, pre-treatment with another agent that reduces the stress response should further enhance apoptosis and the effectiveness of these systemic therapies.

Systemic strategies to reduce the stress response

Systemic strategies that could be used to reduce the endogenous expression of HSPs and inhibit therapy-induced stress responses include anti sense strategies that target HSPs and drug intervention strategies using the flavonoid quercetin or the inhibitor KNK437. The use of quercetin is especially promising.

Quercetin is a broad spectrum inhibitor that interferes with the binding of HSF-1 to heat shock promoters, thereby inhibiting the stress-induced synthesis of HSP mRNAs [41, 42]. Quercetin also is an anti oxidant and a modulator of signalling pathways [43] and is more effective under acidified conditions [44–46]. Quercetin has been shown to significantly sensitize human prostate xenografts to thermal therapy-induced tumour growth delay [47]. Furthermore, the continuous treatment with quercetin over a 5-week period was not toxic to the host nude mice [47]. Separate studies have demonstrated that exposure of prostate tumour cell lines to quercetin or to anti-sense oligonucleotides *in vitro* led to depletion of HSP70 expression and apoptosis in the absence of thermal therapy [48]. Furthermore, pretreatment of the prostate cell lines with quercetin synergistically enhanced apoptosis induced by thermal therapy [48]. Pretreatment with quercetin has also been shown to reduce the induction of HSP70 by the farnesyl transferase inhibitor manumycin in ovarian cancer cell lines and mesothelioma biopsies and to enhance apoptosis [39]. The use of quercetin is translatable to the clinic: quercetin is used to treat chronic prostatitis [49, 50]. Furthermore, there is an increasing literature on the systemic effects of quercetin from the field of clinical nutrition [51, 52].

Less is known about KNK437. It is a compound developed by the Kaneka Corporation (Takasago, Japan). KNK437 inhibits the induction of HSPs *in vitro* and *in vivo* [53, 54].

Pre treatment with KNK437 enhanced thermally induced apoptosis in human cancer cell lines [55, 56].

A different approach to inhibit the stress response involves use of RNA interference technology to knock down specific HSPs. RNA interference is a rapidly emerging and powerful technique used to investigate gene function by degrading a specific mRNA target in a cell or organism and, thus, knocking out or knocking down the level of the encoded protein. The specific mRNA degradation is mediated by complementary double-stranded RNA [57–59]. The use of RNA interference molecules to target HSP70, HSP27 and HSP90 mRNAs may abrogate the therapy-induced stress response. The utility of RNA interference technology for the treatment of cancer remains to be determined.

Impact on the immune response

HSPs are known to enhance and play a role in the immune response elicited against tumour cells (see separate articles in this issue on HSPs and the immune response by Calderwood and Subjeck). Therefore, strategies that decrease treatment induced expression of HSPs may also reduce the immune response directed against the tumours. This cannot be tested using human xenografts, since the rodent hosts (SCID or nude mice) do not have competent immune systems. Strategies that partially but not completely inhibit the treatment induced stress response may allow for increased apoptosis and for an enhanced immune response. Alternatively, strategies that reduce expression of Hsp27 or Hsp70 but not Hsp90 or Hsp110 may also accomplish the same result. Hsp90 [60] as well as Hsp70 [61] have been shown to be expressed on the surface of melanoma cells and are potential immunorelevant targets for immunotherapy [60]. Strategies that target the surface expressed Hsp90 with cell impermeable analogues of geldanamycin also are being considered [33].

Future studies

Future studies require the testing and confirmation of the proposed strategy in multiple tumour models *in vitro* and *in vivo*. Potential agents considered for reduction of the stress response must be capable of being used systemically and/or locally. The potential agents must be capable of reducing the levels of HSPs and inhibiting the induction of HSPs, without inducing normal tissue damage. Studies using rodent models with competent immune systems must also be carried out to determine the effect of inhibiting the stress response on tumour growth delay. A therapeutic gain must be demonstrated *in vivo* before Phase I trials are to be considered.

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References

1. Dewey WC. Failla memorial lecture. The search for critical cellular targets damaged by heat. Radiation Research 1989;120:191–204.
2. Lepock JR. Role of nuclear protein denaturation and aggregation in thermal radiosensitization. International Journal of Hyperthermia 2004;20:115–130.

3. Yatvin MB, Cramp WA. Role of cellular membranes in hyperthermia: Some observations and theories reviewed. International Journal of Hyperthermia 1993;9:165–185.
4. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Natural Reviews in Cancer 2005;5:231–237.
5. Lindquist S, Craig EA. The heat-shock proteins. Annual Reviews in Genetics 1988;22:631–677.
6. Jolly C, Morimoto RI. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. Journal of the National Cancer Institute 2000;92:1564–1572.
7. Jäättelä M. Escaping cell death: Survival proteins in cancer. Experimental Cell Research 1999;248:30–43.
8. Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochemistry & Biophysics Research Communications 2003;304:505–512.
9. Beere HM. 'The stress of dying': The role of heat shock proteins in the regulation of apoptosis. Journal of Cell Science 2004;117:2641–2651.
10. van den Berg AP, Wike-Hooley JL, Broekmeyer-Reurink MP, van der Zee J, Reinhold HS. The relationship between the unmodified initial tissue pH of human tumours and the response to combined radiotherapy and local hyperthermia treatment. European Journal of Cancer & Clinical Oncology 1989;25:73–78.
11. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: A review. Cancer Research 1989;49:6449–6465.
12. Leeper DB, Engin K, Thistlethwaite AJ, Hitchon HD, Dover JD, Li DJ, et al. Human tumor extracellular pH as a function of blood glucose concentration. International Journal of Radiation Oncology, Biology & Physics 1994;8:935–943.
13. Engin K, Leeper DB, Tupchong L, Thistlethwaite A. Tumor pH and response to thermoradiotherapy in superficial human tumors. International Journal of Radiation Oncology, Biology & Physics 1995;29:125–132.
14. Leeper DB, Engin K, Wang JH, Cater JR, Li DJ. Effect of i.v. glucose versus combined i.v. plus oral glucose on human tumour extracellular pH for potential sensitization to thermoradiotherapy. International Journal of Hyperthermia 1998;14:257–269.
15. Han J-S, Storck CW, Wachsberger PR, Leeper DB, Berd D, Wahl ML, et al. Acute extracellular acidification increases nuclear associated protein levels in human melanoma cells during 42°C hyperthermia and enhances cell killing. International Journal of Hyperthermia 2002;18:404–415.
16. Coss RA, Storck CW, Daskalakis C, Berd D, Wahl ML. Intracellular acidification abrogates the heat shock response and compromises survival of human melanoma cells. Molecular Cancer Therapy 2003;2:383–388.
17. Coss RA, Storck CW, Wachsberger PR, Reilly J, Leeper DB, Berd D, et al. Acute extracellular acidification reduces intracellular pH, 42°C-induction of heat shock proteins and clonal survival of human melanoma cells grown at pH 6.7. International Journal of Hyperthermia 2004;20:93–106.
18. Jahde E, Rajewsky MF. Tumor-selective modification of cellular microenvironment *in vivo*: Effect of glucose infusion on the pH in normal and malignant rat tissues. Cancer Research 1982;42:1505–1512.
19. Evelhoch JL, Sapareto SA, Jick DE, Ackerman JJ. *In vivo* metabolic effects of hyperglycemia in murine radiation-induced fibrosarcoma: A 31P NMR investigation. Proceedings of the National Academy of Sciences (USA) 1984;81:6496–6500.
20. Sevic EM, Jain RK. Blood flow and venous pH of tissue-isolated Walker 256 carcinoma during hyperglycemia. Cancer Research 1988;48:1201–1207.
21. Volk T, Jahde E, Fortmeyer HP, Glusenkamp KH, Rajewsky MF. pH in human tumour xenografts: Effect of intravenous administration of glucose. British Journal of Cancer 1993;68:492–500.
22. Jahde E, Volk T, Atema A, Smets LA, Glusenkamp KH, Rajewsky MF. pH in human tumor xenografts and transplanted rat tumors: Effect of insulin, inorganic phosphate, and m-iodobenzylguanidine. Cancer Research 1992;52:6209–6215.
23. Wahl ML, Owen JA, Burd R, Herlands RA, Nogami SS, Rodeck U, et al. Regulation of intracellular pH in human melanoma: Potential therapeutic implications. Molecular Cancer Therapy 2002;1:617–628.
24. Burd R, Lavorgna SN, Daskalakis C, Wachsberger PR, Wahl ML, Biaglow JE, et al. Tumor oxygenation and acidification are increased in melanoma xenografts after exposure to hyperglycemia and meta-iodo-benzylguanidine. Radiation Research 2003;159:328–335.
25. Zhou R, Bansal N, Leeper DB, Glickson JD. Enhancement of hyperglycemia-induced acidification of human melanoma xenografts by inhibitors of respiration and ion transport. Acad Radiologica 2001;8:571–582.
26. Saleh A, Srinivasula SM, Balkir L, Robbins PD, Alnemri ES. Negative regulation of the Apaf-1 apoptosome by Hsp70. Nature Cell Biology 2000;21:476–483.
27. Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, et al. The chaperone function of hsp70 is required for protection against stress-induced apoptosis. Molecular and Cell Biology 2000;20:7146–7159.

28. Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, et al. Hsp27 negatively regulates cell death by interacting with cytochrome c. Nature Cell Biology 2000;2:645–652.
29. Pandey P, Saleh A, Nakazawa A, Kumar S, Srinivasula SM, Kumar V, et al. Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. The EMBO Journal 2000;19:4310–4322.
30. Solit DB, Scher HI, Rosen N. Hsp90 as a therapeutic target in prostate cancer. Seminars in Oncology 2003;30:709–716.
31. Grem JL, Morrison G, Guo XD, Agnew E, Takimoto CH, Thomas R, et al. Phase I and pharmacologic study of 17-(allylamino)-17-demethoxygeldanamycin in adult patients with solid tumors. Journal of Clinical Oncology 2005;23:1885–1893.
32. Goetz MP, Toft D, Reid J, Ames M, Stensgard B, Safgren S, et al. Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. Journal of Clinical Oncology 2005;23:1078–1087.
33. Neckers L, Neckers K. Heat-shock protein 90 inhibitors as novel cancer chemotherapeutics—an update. Expert Opinions in Emergency Drugs 2005;10:137–149.
34. Maloney A, Workman P. HSP90 as a new therapeutic target for cancer therapy: The story unfolds. Expert Opinions in Biological Therapy 2002;2:3–24.
35. Bagatell R, Paine-Murrieta GD, Taylor CW, Pulcini EJ, Akinaga S, Benjamin IJ, et al. Induction of a heat shock factor 1-dependent stress response alters the cytotoxic activity of hsp90-binding agents. Clinical Cancer Research 2000;6:3312–3318.
36. Whitesell L, Bagatell R, Falsey R. The stress response: Implications for the clinical development of hsp90 inhibitors. Current Cancer Drug Targets 2003;3:349–358.
37. Griffin TM, Valdez TV, Mestril R. Radicicol activates heat shock protein expression and cardioprotection in neonatal rat cardiomyocytes. AJP—Heart 2004;287:1081–1088.
38. Cesario A, Catassi A, Festi L, Imperatori A, Pericelli A, Galetta D, et al. Farnesyltransferase inhibitors and human malignant pleural mesothelioma: A first-step comparative translational study. Clinical Cancer Research 2005;11:2026–2037.
39. Hu W, Wu W, Verschraegen CF, Chen L, Mao L, Yeung SC, et al. Proteomic identification of heat shock protein 70 as a candidate target for enhancing apoptosis induced by farnesyl transferase inhibitor. Proteomics 2003;3:1904–1911.
40. Hu W, Wu W, Yeung SC, Freedman RS, Kavanagh JJ, Verschraegen CF. Increased expression of heat shock protein 70 in adherent ovarian cancer and mesothelioma following treatment with manumycin, a farnesyl transferase inhibitor. Anticancer Research 2002;22:665–672.
41. Nagai N, Nakai A, Nagata K. Quercetin suppresses heat shock response by down regulation of HSF1. Biochemistry & Biophysics Research Communications 1995;208:1099–1105.
42. Kim SH, Yeo GS, Lim YS, Kang CD, Kim CM, Chung BS. Suppression of multidrug resistance via inhibition of heat shock factor by quercetin in MDR cells. Experimental Molecular Medicine 1998;30:87–92.
43. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: Antioxidants or signalling molecules? Free Radical Biology Medicine 2004;36:838–849.
44. Lee YJ, Curetty L, Hou Z, Kim SH, Kim JH, Corry PM. Effect of pH on quercetin-induced suppression of heat shock gene expression and thermotolerance development in HT-29 cells. Biochemistry & Biophysics Research Communications 1992;186:1121–1128.
45. Lee YJ, Erdos G, Hou Z, Kim SH, Kim JH, Cho JM, et al. Mechanism of quercetin-induced suppression and delay of heat shock gene expression and thermotolerance development in HT-29 cells. Molecular Cell Biochemistry 1994;137:141–154.
46. Wachsberger PR, Burd R, Bhala A, Bobyock SB, Wahl ML, Owen CS, et al. Quercetin sensitizes cells in a tumour-like low pH environment to hyperthermia. International Journal of Hyperthermia 2003;19:507–519.
47. Asea A, Ara G, Teicher BA, Stevenson MA, Calderwood SK. Effects of the flavonoid drug quercetin on the response of human prostate tumours to hyperthermia *in vitro* and *in vivo*. International Journal of Hyperthermia 2001;17:347–356.
48. Jones EL, Zhao MJ, Stevenson MA, Calderwood SK. The 70 kilodalton heat shock protein is an inhibitor of apoptosis in prostate cancer. International Journal of Hyperthermia 2004;20:835–849.
49. Shoskes DA, Zeitlin SI, Shahed A, Rajfer J. Quercetin in men with category III chronic prostatitis: A preliminary prospective, double-blind, placebo-controlled trial. Urology 1999;54:960–963.
50. Shoskes DA. Treatment response to conventional and novel therapies in chronic prostatitis. Current Urology Reports 2003;4:311–315.
51. Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. American Journal of Clinical Nutrition 2005;81:230S–242S.

52. Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. American Journal of Clinical Nutrition 2005;81:243S–255S.
53. Yokota S, Kitahara M, Nagata K. Benzylidene lactam compound, KNK437, a novel inhibitor of acquisition of thermotolerance and heat shock protein induction in human colon carcinoma cells. Cancer Research 2000;60:2942–2948.
54. Koishi M, Yokota S, Mae T, Nishimura Y, Kanamori S, Horii N, et al. The effects of KNK437, a novel inhibitor of heat shock protein synthesis, on the acquisition of thermotolerance in a murine transplantable tumor *in vivo*. Clinical Cancer Research 2001;7:215–219.
55. Nonaka T, Akimoto T, Mitsuhashi N, Tamaki Y, Yokota S, Nakano T. Changes in the localization of heat shock protein 72 correlated with development of thermotolerance in human esophageal cancer cell line. Anticancer Research 2003;23:4677–4687.
56. Ohnishi K, Takahashi A, Yokota S, Ohnishi T. Effects of a heat shock protein inhibitor KNK437 on heat sensitivity and heat tolerance in human squamous cell carcinoma cell lines differing in p53 status. International Journal of Radiation Biology 2004;80:607–614.
57. Dykxhoorn DM, Novina CD, Sharp PA. Killing the messenger: Short RNAs that silence gene expression. Natural Reviews in Molecular Cell Biology 2003;4:457–467.
58. Bedford JS, Liber HL. Applications of RNA interference for studies in DNA damage processing, genome stability, mutagenesis, and cancer. Seminars in Cancer Biology 2003;13:301–308.
59. Betz N. Technically speaking: RNAi: RNA interference. Promega Notes 2003;83:33–36.
60. Becker B, Multhoff G, Farkas B, Wild PJ, Landthaler M, Stolz W, Vogt T. Induction of Hsp90 protein expression in malignant melanomas and melanoma metastases. Experimental Dermatology 2004;13:27–32.
61. Farkas B, Hantschel M, Magyarlaki M, Becker B, Scherer K, Landthaler M, Pfister K, Gehrmann M, Gross C, Mackensen A, Multhoff G. Heat shock protein 70 membrane expression and melanoma-associated marker phenotype in primary and metastatic melanoma. Melanoma Research 2003;13:147–152.