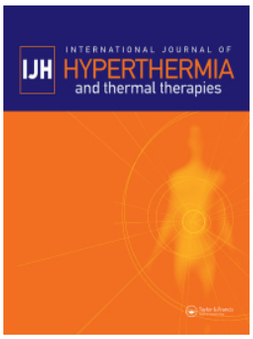

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Role of HSPs and telomerase in radiotherapy

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Abstract
The inability of radiotherapy to control tumour growth is still a daunting clinical problem leading to failure of the overall treatment regimens. The fundamental question is; could tumour cells be specifically sensitized to ionizing radiation (IR) by heat or factors exclusively expressed in tumour cells? One such factor, expressed in most tumours and silent in somatic cells, is telomerase. Biochemical and genetic studies have established an association between telomere maintenance and extended life span of human cells mediated through the expression of the catalytic sub-unit of telomerase (hTERT). Because of this, telomerase is an attractive target for inhibition in anti-cancer therapy. Telomeres are maintained by telomerase and hTERT interacts with heat shock protein (HSP) chaperones. This review will focus on the possible role of HSPs and telomerase in sensitizing tumour cells and, thus, enhancing the potential of targeted radiotherapy.

Keywords: *Telomeres, telomerase, HSP70, HSP90*

Introduction

Hyperthermic cell stress activates a highly conserved programme of rapid alterations in normal cellular metabolism to optimize synthesis of a limited, specific set of proteins known as HSPs. The most highly induced and conserved HSPs in all organisms from *Escherichia coli* to man is HSP70 [1]. The evolutionary conserved members of the HSP70 family prevent the disruption of normal cellular processes that involve mitosis, meiosis or differentiation by environmental stressors [2, 3]. Members of the HSP70 family play essential roles in preventing misfolding and aggregation of newly synthesized or unfolded proteins [4–6]. HSP70 holds unfolded substrates in an intermediately folded state to prevent irreversible aggregation and then catalyzes the refolding of unfolded substrates in an energy- and co-chaperone-dependent reaction. HSP70s interact with co-chaperones through the N-terminal ATPase domain and with substrates at the C-terminal substrate domains.

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Co-ordinated binding and release of substrates by these molecular chaperones is strictly dependent on their ATPase activity. Several studies have suggested a role for HSPs during development; however, limited information is available as to whether inactivation of such genes could influence genomic stability. It has been shown that HSP70 binds to human apurinic/apyrimidinic endonuclease (HAP) and enhances the specific endonuclease activity of HAP1, supporting the idea that HSP70s have a role in the repair of DNA damage [7]. Brondani et al. [8] have suggested that HSP70 protection against IR-induced apoptosis might underlie glioblastoma radioresistance. This study developed a mouse model system to determine whether the inactivation of HSP70 influences the genomic stability and DNA repair after heat and IR treatment [9]. Hsp70.1 and Hsp70.3 are the only known HSPs that are heat induced in mice [10–12]. These two genes are identical and their functions are thought to be redundant. Because of this redundancy, mice in which both Hsp70.1 and Hsp70.3 were knocked out allowed one to then establish cell lines deficient for HSP70.

Interaction between HSPs and the catalytic unit of telomerase

Telomerase, as a key determinant of telomere homeostasis, is subject to tight regulation during tissue development, organogenesis, cellular ageing and tumourigenesis. It is activated ubiquitously in the human embryo, down-regulated in most of the somatic cells and reactivated during tissue repair and tumourigenesis. Telomerase is a cellular ribonucleoprotein reverse transcriptase that can maintain the length of telomeres by using its integral RNA component as a template for the addition of TTAGGG repeats onto the 3′ end of linear chromosomes. The activation of telomerase leads to unlimited cell proliferation and is believed to play a critical role in neoplastic transformation [13–15]. The human telomerase holoenzyme contains a catalytic protein component, hTERT, and a 451-base integral RNA, human telomerase RNA (hTR), which are essential for assembling telomerase activity *in vitro* and *in vivo* [16]. The reverse transcriptase motifs of the protein component of telomerase are conserved among diverse organisms. Several human chaperone proteins have been found associated with hTERT. HSP70 has been found to be associated with the catalytic unit of telomerase (TERT) prior to its assembly with the RNA component of telomerase (TR); however, p23 and HSP90 are important for the assembly of telomerase activity *in vitro* as well as *in vivo* [17]. Kim et al. [18] reported that disruption of HSP90 by geldanamycin promotes efficient ubiquitination and proteasome-mediated degradation of hTERT. It is likely that HSP70 may be important for the stability of hTERT prior to its assembly into a functionally active telomerase complex. Barker et al. [19] reported a link between the telomerase activity and HSP70 expression. They found that the autonomous cells constitutively expressed telomerase, whereas the non-autonomous cells expressed telomerase activity only transiently [19]. Interestingly, northern analysis of HSP70 indicated that, like telomerase, *HSP70* gene expression was constitutive in autonomous cells and transient in non-autonomous cells [19]. These results suggest that hTERT expression may partly be regulated by heat shock elements. Heat shock transcription regulatory elements have been identified in the telomeric sequences in *Chironomus thummi* [20]. Therefore, comparison of telomerase activity in cells with and without HSP70 was carried out [9].

Inactivation of HSP70 influences telomerase activity

While HSP70 associates with TERT, such an association is not required for the functional activity of hTERT [21]. However, the inactivation of HSP70 does influence TERT activity [9]. Cells derived from knock out *HSP70* mice (Hsp70.1/3$^{-/-}$) had significantly lower

levels of telomerase activity as compared to controls (Hsp70.1/3$^{+/+}$) [9]. Ectopic expression of Hsp70.1 in Hsp70.1/3$^{-/-}$ mouse cells restored telomerase activity to similar levels of activity present in parent cells. Such results do not demonstrate that HSP70 is essential for telomerase activity, as Hsp70.1/3$^{-/-}$ cells do have telomerase activity but at reduced levels. These results demonstrate that HSP70 may be required either for the stability of the telomerase complex or for the formation of such a complex required for enzymatic activity. Recent studies have revealed that, beyond the role of telomerase in telomere maintenance, telomerase provides additional functions in DNA repair and cell survival [22]. Further studies are needed to determine whether or not HSP70s play any role in the assembly of the telomerase complex or its stability.

Inactivation of Hsp70.1/3 results in telomere instability

Recent studies revealed that ectopic expression of TERT protein is associated with enhanced HSP70 expression along with genome stability and DNA repair [22]. Such observations support the argument that HSP70 may a have role in the stability of telomeres. Analysis of telomeres by using fluorescent *in situ* hybridization (FISH) for telomeric repeats on metaphase did not reveal any significant overall changes in signal intensities in cells with and without Hsp70.1/3. However, there was a slightly higher proportion of chromatid ends ($\sim$10% of telomeres per metaphase), which had less telomere specific fluorescent signals when compared to the parental cells ($\sim$3% of telomeres per metaphase) [9]. Loss of telomeric signals has been linked with chromosome end-to-end associations. Hsp70.1/3$^{-/-}$ had $\sim$0.55 chromosome end-to-end associations per metaphase whereas Hsp70.1/3$^{+/+}$ cells displayed 0.16 chromosome end-to-end associations per metaphase [9]. Since chromosome end-to-end associations may lead to anaphase bridge formation, the same cells were analysed for anaphase bridges by omitting the colcemid treatment. Hsp70.1/3$^{-/-}$ cells displayed a 2.5-fold higher frequency of anaphase bridges compared to parental cells. Telomeric signals were seen at most of the fusion sites, indicating that total loss of telomeres is not required for the formation of chromosome end-to-end associations in these cells [9]. Chromosome end-to-end associations have been linked with chromosome aberrations. Hsp70.1/3$^{-/-}$ mouse embryonic fibroblast (MEF) cells displayed a higher frequency of chromatid as well as chromosome type aberrations when compared to Hsp70.1/3$^{+/+}$ MEF cells [9].

Hsp70.1/3 inactivation effects heat- and IR-induced cell killing and chromosomal repair

The differences in telomerase activity in Hsp70.1/3$^{-/-}$ and Hsp70.1/3$^{+/+}$ MEF cells correlated with detected differences in doubling times and spontaneous chromosome damage [9]. Such results suggest that Hsp70.1/3 would affect cell survival and the ability to repair DNA damage. Consistent with the differences in population doubling times, Hsp70.1/3$^{-/-}$ cells exhibited enhanced cell killing by IR treatment compared to parental cells [9]. Heat treatment again had a significant effect on IR-induced cell killing for Hsp70.1/3$^{-/-}$ cells, suggesting a critical role of Hsp70.1/3$^{-/-}$ in the heat-modulated IR-induced cell kill [9]. Ectopic expression of Hsp70.1 in Hsp70.1/3$^{-/-}$ cells rescued heat-and IR-sensitivity for cell kill. Thus, inactivation of HSP70 influences telomerase activity, telomere stability and cell survival after heat- and or IR-treatment, cellular phenotypes that could be linked to defective chromosomal repair.

One way to address whether DNA repair is affected by inactivation of Hsp70.1/3 in these cells is to compare cell-cycle-stage specific chromosomal aberrations in fibroblasts with and without Hsp70.1/3. Cell cycle phase-specific chromosome aberrations can be

ascertained based on the frequency of chromosomal and chromatid type aberrations observed at metaphase. G1-specific aberrations detected at metaphase are mostly of the chromosome type. S-phase type aberrations detected at metaphase are chromosomal as well as chromatid type. G2-type aberrations detected at metaphase are predominantly the chromatid type. No major induction of chromosome aberrations in G1-phase cells with or without Hsp70.1/3 was reported when they were treated with heat at 43°C for 30 min then replated 9 h after heat treatment and aberrations scored at metaphase [9]. When cells were treated with heat at 43°C for 30 min and then immediately exposed to 3 Gy of IR, Hsp70.1/3$^{-/-}$ cells had relatively higher G1 type chromosome aberrations as compared to Hsp70.1/3$^{+/+}$ cells; however, such differences were not statistically significant [9]. In contrast to G1, S-phase cells of Hsp70.1/3$^{-/-}$ cells displayed lower mitotic indices and higher frequencies of chromatid and chromosomal aberrations than parental cells after heat and IR treatment [9]. These observations establish that the inactivation of Hsp70.1/3 influences S-phase specific chromosomal repair. No major difference for G2-phase specific chromosome aberrations after heat and IR treatment was observed between Hsp70.1/3$^{-/-}$ and Hsp70.1/3$^{+/+}$ cells. Such results suggest that the Hsp70.1/3$^{-/-}$ gene products might have a specific role in the repair of DNA during S-phase of the cell cycle.

Perspectives

The undesirable sequelae of treatment are a necessary limiting by-product of aggressive IR treatment focused on the goal of eliminating the tumour. Hyperthermia in combination with IR is considered an essential tool in the treatment of urological and some other cancers to make cells more sensitive to cell kill by IR. There is a need to find the biochemical differences between normal and tumour cells which could be exploited for targeted therapy. As heat induces HSP70 and telomerase is specifically present in most tumours [23], it is important to understand whether heat influences telomerase function. If heat abrogates telomerase function, then this may be a mechanism by which heat could specifically sensitize tumour cells to enhanced cell killing by IR. Sun et al. [24] have reported that the catalytic activity of telomerase increased whereas the processivity of telomerase decreased as temperature (up to 37°C), concentrations of dGTP, primer and K+ were increased in an *in vitro* assay. Direct inhibition of telomerase by heat seems to be a remote possibility as heat treatment enhances the telomerase activity (unpublished data). However, it is possible that the accessibility of the telomerase to its target site may be affected by heat because of alterations in telomere chromatin structure or nuclear matrix associations. Direct inhibition of telomerase using peptide nucleic acid (PNA), locked nucleic acid (LNA) and 2′-O-MeRNA oligomers resulted in progressive telomere shortening and subsequent apoptosis [25, 26]. Since ectopic expression of hTERT leads to transcriptional alterations of a sub-set of genes, genome stabilization and an increase in ATP levels [22], highlights the importance of the potential significance of targeting hTERT as an anti-cancer therapy agent. These novel functions of telomerase are distinct from its known effect on telomere length and have potentially important biological consequences. Thus, to study the effects of heat on telomerase, will help one to understand whether heat influences any of the novel function of hTERT (described above) [27]. In tow, these studies should provide significant information that has implications for heat-induced radiosensitization.

Thus, cells that are positive for telomerase activity may become more sensitive to cell kill by heat followed by IR. Since inactivation of both HSP70 influence spontaneous chromosome damage, telomerase activity, telomere stability, IR and heat modulated

IR-induced cell kill and chromosome repair. Hyperthermia is known to enhance cell kill by IR and hTERT expression extends the lifespan of the cells, it will be important to understand whether knockdown of telomerase activity will enhance heat modulated IR-induced cell killing. Future studies needs to focus on how HSP70 as well as hTERT can be down regulated prior heat and IR exposure to enhance the cell killing of tumour specific cells.

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