cancer (5 years 2 months, 5 years 6 months), and both had tumours with abundant pS2 gene expression. We, therefore, agree with Cappelletti and colleagues [10] that considerable caution must be exercised before acceptance of pS2 as a prognostic indicator of patient survival in breast cancer, particularly bearing in mind the well-known heterogeneity of these cancers at the cellular level.

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Feature Articles

Cancer and the Heat Shock Response

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HEAT SHOCK proteins (HSP) represent one of the most conserved groups of proteins throughout evolution [reviewed in Refs 1 and 2]. They have been found in all organisms examined to date including prokaryotes, yeast and plants, as well as higher eukaryotes. Although first identified in response to heat shock (HS), the wide range of stimuli able to cause induction (including oxidative injury, sodium arsenite, heavy metals, amino acid analogues and serum deprivation) has led to the concept that HSPs are part of a larger group of "stress proteins". Many members of HSP families are also expressed under normal conditions in a cell-cycle dependent manner [2, 3].

Both under normal conditions and in response to stress, HSPs are implicated in protein–protein interactions such as folding, translocation and prevention of inappropriate protein aggregation [1, 4]. Many features of the functions, regulation and expression of HSP suggest they play a role in cancer (Figure 1). In this review, we address the biological aspects of HS, i.e. HSP expression in tumours, their involvement in apoptosis, interactions with proto-oncogenes and *TP53*, as well as their role as tumour antigens and the clinical aspects of HS, such as its use in cancer therapy (hyperthermia).

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THE HS RESPONSE

Introduction: classification of HSPs and possible roles in cancer

The HSPs are classified on the basis of their molecular weight as determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). There are five main families: low molecular weight, hsp65, hsp70, hsp90 and hsp100. Each family is comprised of

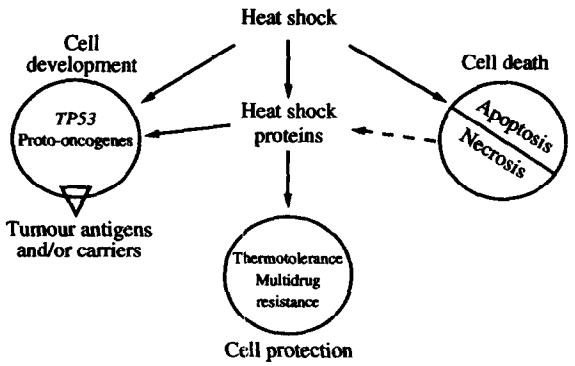

Figure 1. Heat shock, heat shock proteins and their involvement in cancer and cancer therapy.

several members, with similar molecular weights, but different patterns of induction and expression.

Not all the HSPs are thought to be involved in cancer. At present, it would appear that only some members of the hsp27, hsp70 and hsp90 families play a defined role in cancer (summarised in Table 1). HSP expression, in response to physiological and pathophysiological conditions, is accentuated in response to both cell proliferation and conditions prompting cell death (apoptosis). This may be significant as several proto-oncogenes are also induced by these stimuli, and HSPs may play a role in stabilising the mRNA for these genes.

As indicated in Table 1, the family of low molecular weight proteins, particularly hsp27, may serve as intrinsic markers of tumour cells, different patterns of phosphorylation relating to resistance or susceptibility to chemotherapeutic drugs. The recent discovery of HSPs from the hsp70 and hsp90 families on the surfaces of tumour cells, where their presence may have immunological consequences, has suggested possible roles as tumour antigens or tumour antigen carriers. Indeed, HSPs are antigenic determinants for subsets of T-cells of both $\alpha\beta$ and $\gamma\delta$ origin. Therefore, it is possible that hyperthermia, by inducing membrane expression of HSPs, is effective not only by causing cell death, but also by increasing the antigenicity of the tumour and thus the natural immunological response. According to their major cellular function as molecular chaperones. HSPs may also serve as carriers for antigenic tumour peptides and/or chaperone oncogenes essential for the regulation of cell proliferation.

Regulation

The HS genes are found throughout the chromosomal pool (illustrated by the dispersal of the hsp70 genes throughout the genome of several animals [5, 6]). Expression of all the HS genes is mediated through specific HS transcription factors (HSF-1 and HSF-2), which are found as inactive monomers (possibly bound to members of the hsp70 family) in the cytoplasm prior to induction. Following HS or a number of other stresses, HSF-1 trimerises and relocates to the nucleus where it interacts with a specific binding site, the HS element (HSE), a highly conserved binding site, with the consensus sequence-GAA-TTC-found in all HS and some related genes [7]. HSF-2, which is not heat-inducible but is activated by other stresses such as hemin, also trimerises and relocates to the nucleus, where it too interacts with the HSE, but the two factors have different binding affinities.

Localisation

The localisation of HSP to the surface of tumour cells, in contrast to their normal intracellular location, suggests a possible role as markers of tumour cells [8, 9]. However, since no polymorphism or structural differences have been observed between HSPs present in or on tumour cells and those expressed by normal cells, it was suggested by Srivastava and Heike [10] that HSPs may not be tumour antigens *per se*, but instead may be involved in antigen presentation (see below).

HSP EXPRESSION IN CANCER

The search for recognition markers which identify tumour cells specifically is a priority if one wishes to eliminate tumour cells without harming nearby normal cells. The latest candidates for these so-called "tumour antigens" are the HSPs. Tumour cells have been reported as having increased HSP levels, and unusual expression patterns of HSPs are seen in tumours of both mouse and human origin [8, 9]. The presence of HSPs on the surface of tumour cells indicates that they could serve as a possible clinical marker. However, it is not yet clear how HSPs get to the cell surface, since they have no recognised signal sequence. The possibility that they are released by adjacent dying cells and absorbed on to the surface of intact cells has yet to be addressed.

Members of the hsp90 family were the first to be implicated as tumour antigens [11]. A cell-surface tumour-specific transplantation antigen, which promotes protective immunity, has

Table 1. HSP families and their proposed roles in cancer and/or cancer therapy

Protein family	Proposed role in cancer	References
Low molecular weight	Phosphorylation isoforms of hsp27 may be a molecular general marker of a transformed cell, and may be responsible for some of the drug resistance and thermotolerance seen in transformed cells.	1
	Overexpression is linked with a poor prognosis in breast cancer, but an increased chance of recovery in malignant fibrous histiocytoma (MFH).	14, 15
hsp70	Interacts with misfolded or unfolded proteins, as well as proto-oncogenes and the tumour suppressor gene, *TP53*.	1, 35, 38
	Present on tumour cells' surface, possible role in antigen presentation?	20–23
	Involved in thermotolerance and drug resistance.	13
	Induced by some stimuli which lead to apoptosis.	23
hsp90	Increased expression in some breast cancers.	12
	Role as a tumour antigen.	10, 11
hsp100	Possible role in thermotolerance.	1

significant sequence homology to the hsp90 from yeast and hsp83 from *Drosophila melanogaster* [11]. Subsequently, elevated levels of a constitutive member of this family (hsp89α) have been found in malignant breast tissue [12]. However, despite the hormone-dependent nature of many breast tumours and the association of steroid receptors with hsp90 family members, no correlation between levels of hsp89α and the oestrogen receptor has been seen [12]. Elevated levels of another member of this family (hsp90α) are also seen in some leukaemias, but the significance of this has yet to be established.

Whilst levels of hsp70 expression may be useful as a diagnostic tool in the study of breast cancer [13], the most important HSP in this condition would appear to be hsp27, levels of which may define the chances of successful treatment. Increased levels of hsp27 are found in a number of cancers including breast cancer and leukaemias (for review see [14] and refs therein; [15]). The hsp27 present in tumour cells, and that observed in normal cells are identical, except for their patterns of phosphorylation. Two isoforms of hsp27, neither of which is found constitutively, and which differ only in their phosphorylation patterns have been found in acute lymphoblastic leukaemia cells. High expression of hsp27 in breast cancer is connected with a poor survival rate [14], and *in vitro* studies show increased drug resistance with overexpression of hsp27 [16]. In contrast, patients with malignant fibrous histiocytoma, who express high levels of hsp27, have a greater chance of recovery, and no hsp27-dependent drug resistance is observed [14]. The possibility of cancer-related and tumour-specific phosphorylation patterns, and functions of hsp27 deserves further investigation.

The possible role(s) of HSPs in tumour antigenicity are several (Figure 2). Firstly, altered HSPs on the surface of tumour cells may function as tumour antigens in a T-cell mediated manner, as this expression does not promote an antibody response, or they may be presented in the context of major histocompatibility complex (MHC) class I or II (Figure 2). Secondly, HSPs may facilitate the presentation of tumour-specific peptides by MHC molecules. Thirdly, Srivastava and Heike [10] proposed that

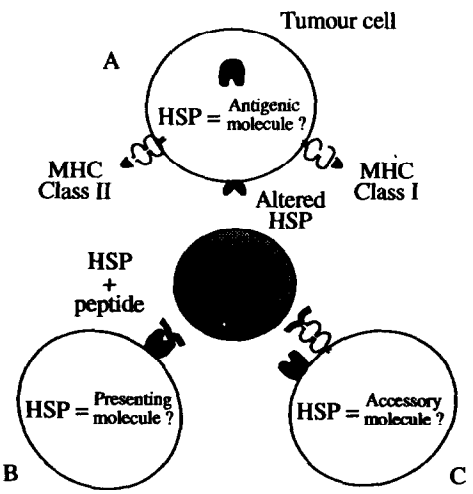

Figure 2. Possible mechanisms by which HSP and T-lymphocytes interact. HSPs may be altered due to cellular transformation and be presented by MHC class I (A) on the surface of tumour cells, where they interact with T cells expressing the αβ or γδ T-cell receptors. Alternatively, HSPs may function as antigen-presenting molecules in a manner similar to MHC class I or II (B). HSPs may also act as accessory molecules for antigen presentation (C). Adapted from [80].

HSPs act as carriers of peptides in a manner similar to that of the MHC. Preparations of a 96 kDa HSP and hsp70 both induce protective immunity against the tumour from which they were initially prepared [17, 18]. The protection seems to be T-cell mediated with both CD4+ and CD8+ T-cells displaying cytotoxicity in *in vitro* studies. On closer examination, it appears that both preparations contained antigenic peptides which had co-purified with the HSPs, possibly because they were associated *in vivo* in a manner similar to that of peptides with MHC class I or II. This may be explained by recent work on the structure of hsp70, which suggests the presence of a peptide binding groove resembling that found within the MHC molecules [19]. Several members of the hsp70 family may actually be involved in the various stages of antigen processing and presentation [20–22].

HSP and apoptosis

The increased expression of HSPs in tumours may have implications in other biological processes including apoptosis. The development of a tumour is dependent on the intricate balance between the rate of cell proliferation and the rate of cell death. Agents which cause tumour growth can do so in two ways: either a stimulation of cell proliferation or a decrease in the rate of programmed cell death (PCD) or apoptosis (Kerr and Harmon in Ref. [23]). Tumour cells may possess an increased level of PCD, but this is outweighed by the increased cell proliferation or decreased cell death in other parts of the tumour. Although HSPs are synthesized during cell proliferation, many of the stimuli which cause cell death also induce HSP expression. The expression of HSPs at this stage may be part of cellular defence mechanisms or of the developmental programme [24].

The concept of cell death as an important part of development was slow to gain acceptance, but is now recognised as essential in many stages including embryonic and neuronal development, and the clonal selection of lymphocytes [23, 25]. Apoptosis is an active process, which occurs via a series of distinct morphological changes including cell shrinkage, membrane blebbing, chromatin condensation and DNA fragmentation [23]. Unlike necrosis, it does not cause an inflammatory response. Cells dying from apoptosis are phagocytosed by neighbouring cells without releasing their contents. The trigger for apoptosis may be a sudden increase in intracellular calcium, resulting from an influx, a redistribution of internal calcium stores or a combination of both [24]. This increase in calcium levels triggers a calcium-dependent endonuclease leading to DNA fragmentation. The active nature of apoptosis requires protein synthesis, and similar proteins are induced during both cell proliferation and during apoptosis. These include the immediate early genes (IEG) C-*MYC* and C-*FOS*, as well as hsp70 [24]. The induction of the IEGs, such as C-*FOS* and C-*MYC*, both of which are thought to play important roles in apoptosis [26, 27], may be in response to the increased intracellular levels of calcium via protein kinases other than protein kinase C. The induction of apoptosis by C-*MYC* may be a target of the proto-oncogene *BCL-2*, the interaction of *BCL-2* with C-*MYC* preventing apoptosis [28, 29]. Possible links between HSPs and *BCL-2* include their antioxidant and protective functions, and prevention from apoptosis. HSPs and apoptosis share modulation by similar second messengers, which are being further investigated in our laboratory, whereas mitochondria may represent important targets for the protective effects of both ([30]; Polla and associates, in preparation). Overexpression of hsp70 (possibly the constitutive form, hsc70) may also protect from necrotic cell death induced by tumour necrosis factor (TNF)-α and -β [31, 32].

HSP, proto-oncogene products and TP53

The HSPs may play an indirect role in the development of cancer through their ability to interact with normal and mutant cellular proteins. They are known to bind unfolded or denatured proteins, either to assist in refolding, or as a guide to the lysosomes for degradation. Recent reports show that HSPs are able to interact with many of the proteins implicated in cancers, including products of the proto-oncogenes and the tumour suppressor gene, *TP53*.

HSP and proto-oncogene products

The products of proto-oncogenes, the cellular homologues of viral genes implicated in some cancers, are able to interact with HSPs. Like HSPs, they are expressed rapidly and transiently in response to diverse stimuli, including HS [33], which also causes a significant increase in the stability of C-*FOS* and C-*MYC* mRNA, suggesting a role for regulation at both the transcriptional and post-transcriptional level [34]. The proto-oncogenes have been implicated in the expression of hsp70 itself. In particular, c-*myc* can activate the *Drosophila* hsp70 promoter [35] and the human hsp70 gene, but in a manner distinct from basal expression or the induction by HS, serum or viruses. The presence of two sequences in the HSP promoter region able to bind C-*MYC* suggests that C-*MYC* or a protein complex containing C-*MYC* may play a role in hsp70 expression by direct interaction with the promoter [36]. Hsp70 is not the only HSP able to interact with the proto-oncogene products: indeed, hsp90 can also form a stable complex with the oncogenic protein-tyrosine kinase pp60v-src. This interaction occurs during or just after synthesis of pp60v-src and the interaction is specific for pp60v-src. Hsp90 has several effects on pp60v-src, including stabilisation of the protein, suppression of kinase activity during its passage to the membrane and modulation of kinase activity and specificity [37].

TP53

Mutations and/or deletions of one allele of the tumour suppressor gene, *TP53*, and deletion of the second allele are now recognised as the most common genetic lesion found in human cancer [38–40]. *TP53* negatively controls cell cycle, and tumour formation is the result of loss-of-function mutations [41]. The mutations would appear to be both tissue- and cancer-specific, non-random, and they are often point mutations clustered in four of the five coding regions [39, 41]. Many mutants of mouse and human p53 undergo a common conformational change [42], which leads to an increased half-life in most instances, compared with the 20 min half-life observed for wild-type [43].

The stabilisation of p53 in SV40 transformed cells appears to be the result of its association with the large T antigen, or, in human tumours, a change in the conformation of the protein [42]. Interactions between p53 and hsp70 have also been proposed to stabilise mutant *TP53* [43–45]. The ability to bind hsp70 (not possessed by wild-type [WT] p53 [43]) is found only in mutant p53 mutants with specific mutations [46a]. p53 mutated in its central region may expose the hydrophobic domains present in this region, leading to recognition and binding by the chaperone [46b]. Though a highly conserved domain in the amino terminus of mutant p53 proteins, which may serve as a possible hsp70-specific binding site, has been isolated [46b], it appears that the binding of mutant p53 and hsp70 may involve the whole protein [47]. A dimer involving mutant and WT p53 can be formed in certain transformed cells, possibly resulting in inhibition of the normal functioning of WT p53 [48]. This transforming ability is reduced by overexpression of hsp70 possibly by preventing mutant-WT interaction [49]. Some of these interactions between p53 and hsp70 are depicted in Figure 3.

Furthermore, WT p53 is able to down-regulate hsp70 expression by interacting with the CCAAT-binding factor (CBF), whilst the mutant form is devoid of this activity [50]. Indeed, the p53 protein can play a dual role in transcription. p53 contains a strong transcriptional sequence near its amino terminus, and Kern and colleagues identified specific DNA sequences able to bind p53 [51]. Although these sequences are not specific promoter regions, this observation suggests that p53 can regulate transcription directly. p53 also interacts with other transcription factors, including the TATA binding protein and the CBF. Other genes, whose transcription is affected by p53, include the proto-oncogene, C-*FOS*. Here, WT, but not mutant p53, is able to down-regulate the transcription of C-*FOS* [52]. This repression seems to be through interference with the C-*FOS* basal promoter activity rather than with a particular element therein. WT p53 is also able to down-regulate the expression of the MDR1 gene, and this effect is reversed by mutants of p53 [53].

High levels of mutant p53 protein have been found in over 25% of human breast cancers, and some patients possess anti-p53 antibodies. In lung and perhaps other cancers, the development of antibodies depends on the type of p53 mutations [54]. Davidoff and coworkers [55] found that most if not all antibody-eliciting tumours possess mutant p53–hsp70 complexes, suggesting a role for hsp70 in the antigenic presentation of p53, whereby hsp70 assists in translocation of mutant p53 from the cell nucleus to the surface membrane, where the mutant protein is either released or plays an immunogenic role. From these models, one would predict that patients possessing p53–hsp70 complexes and hence antibodies to p53 would have a better prognosis but the opposite is true: survival at 5 years is better for patients without circulating antibodies to p53 for reasons yet to be revealed (Soussi and Peyrat, submitted).

Overall, these results suggest that p53 is able to influence gene expression in both a positive and negative manner, sometimes within the same gene. This implies a complex regulation process of many genes by p53, the subtlety of which was not previously appreciated.

Clinical use of heat shock

Heat alone can kill cells in a temperature- and dose-dependent manner. The rate of cell death in human cells increases exponentially as a function of length of exposure to given temperatures above 42°C. Cell death occurs via two distinct mechanisms which are dependent upon the severity of the treatment. Mild hyperthermia, such as 1 h at 43°C, promotes cell death through apoptosis [56], while increasing either the temperature or the duration of exposure or both leads to cell death via necrosis [57]. The initial targets of hyperthermic cell death are likely to be the plasma membrane and integrated proteins, but many other structural and functional elements (including mitochondria and nuclei) are affected by heat, and it has been difficult to establish the primary event leading to cell death [58].

History

Hyperthermia and fever therapy have been empirically used as effective treatments for many diseases over centuries. Hyperthermia as a treatment was first mentioned in the Edwin Smith surgical papyrus in the 17th century BC and was well known by

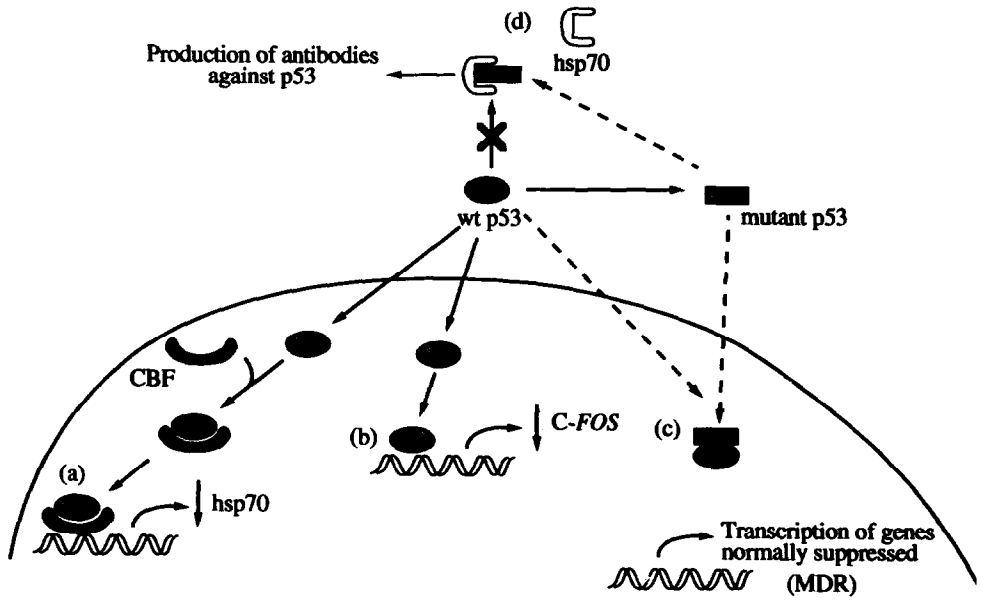

Figure 3. The interactions of wild-type and mutant p53 with each other and hsp70. Wild-type (WT) p53 enters the nucleus and interacts with the CCAAT binding factor (CBF) causing down-regulation of hsp70 (a), or binds to the promoter region directly, resulting in the up-regulation of hsp70 gene expression, but the down-regulation of other genes, including C-*FOS* and MDR1 (b). WT and some mutant (Mut) p53 form a complex which drives WT p53 into the mutant conformation and prevents its binding to DNA (c). The binding of Mut p53 to hsp70 may inhibit this complex formation (d). WT p53 does not bind hsp70.

physicians of ancient Egypt and Greece. Hyperthermia has been used in dermatology to treat diseases such as syphilis and psoriasis [59]. Its potential use as a therapeutic strategy in the treatment of cancer was first demonstrated by Crile [60], three decades ago, when he showed that at least some melanomas implanted in the paws of mice regressed, without damage to the surrounding tissue, when treated with heat.

Mechanisms of heat-induced killing

One mechanism which is known to cause heat-induced cell death occurs as a result of polyamine catabolism (Carper and associates in Ref. [61]). HS induces several enzymes responsible for the catabolism of polyamines. Byproducts of these reactions include reactive oxygen species such as hydrogen peroxide (H_2O_2). The cytotoxicity of polyamine catabolism may, at least in part, be due to this H_2O_2, as cell death can be inhibited by the addition of catalase [62]. Other products of polyamine catabolism such as ammonia, may also contribute to the observed cytotoxicity [62].

Some cells within a tumour may be more susceptible to heat not—as was first thought—because they are transformed, but rather by the nature of their physical environment which is often nutrient deficient, hypoxic and at a low pH [2]. The observation that tumour cells in late S-phase, whilst being resistant to radiation are more sensitive to hyperthermic death, suggested a possible role for combined treatments in increasing the efficacy of anticancer regimens. Numerous studies report encouraging clinical effects of hyperthermia in cancer therapy. Overgaard's review [63] of papers up to 1984 revealed that 213 studies had been published and over 11 000 patients treated. Many of the studies indicate a decrease or stabilisation of tumour growth, particularly with superficial tumours, using hyperthermia in combination with either radiation and/or chemotherapy in patients with advanced disease.

Techniques

In order for hyperthermia to become more widely used in cancer therapy, a number of problems of either a technical or treatment-induced nature have had to be addressed in recent years. The heterogeneous nature of the tumour with its varied blood supply means that not all areas of the tumour are heated to the same temperature. Several techniques are used for whole body (WBH) and regional hyperthermia (RHT), including perfusion, ultrasound and electromagnetic waves [64]. Hyperthermic isolated limb perfusion (HILP) has been successful in the treatment of metastatic melanomas of the limb, where only the affected areas were perfused. Complete responses in measurable disease are reported in approximately 80% of patients by several studies [65], but the duration of locoregional disease control is still rather short, lasting only 3–14 months. Long-term survival rate is not significantly increased due to the occurrence of systemic diseases; according to the stage of the disease, only 25–58% of patients survived for 5 years [66].

Single microwave applicators for superficial tumours or multiple applicators (such as the annular phased array system or APAS) for deep-seated tumours are the most common techniques used for RHT [64]. However, it has been difficult to measure the temperature distribution induced by treatment within the tumour and surrounding tissue [64].

Results

Today RHT is being used only in combination with more traditional techniques, such as radiation or chemotherapy. The combination of radiotherapy with hyperthermia has been particularly successful in the treatment of superficial tumours. When the response rate of superficial tumours treated either with hyperthermia and radiation or by radiation alone were compared, complete regression was noted in 63.6% of the combined treatment, but in only 18% with radiation alone [65]. The results of

the European Society of Hyperthermic Oncology phase III study for metastatic malignant melanoma (ESHO 3-85) show that hyperthermia significantly improves the therapeutic effect of radiotherapy [67].

Combined therapy (hyperthermia plus chemotherapy and/or radiotherapy)

Hyperthermia combined with systemic full-dose standard chemotherapy has been more recently introduced as a clinical strategy. The combination of anticancer drugs with heat treatment can increase efficacy for a number of reasons: the rate of drug uptake into the tumour cell, its intracellular distribution and the rate of metabolism are all affected by temperature increases [68]. Some, but not all of the common chemotherapeutic drugs (including bleomycin and doxorubicin) seem to have increased efficacy at higher temperatures. Others, particularly those rich in sulphydryl groups, which exhibit no cytotoxicity at physiological temperatures, become toxic at temperatures of 43°C and above [69]. The combined treatments have shown increased success in many tumours including adenocarcinomas, colorectal cancer, malignant melanomas, sarcomas and squamous cell carcinomas [64, 68, 70]. The treatment of peritoneal cancer by continuous hyperthermic peritoneal perfusion (CHPP) with mitomycin-C resulted in a survival rate after 30 months of 83% of patients compared with 67.3% in the controls [71]. One of us (RI) has recently reported encouraging results using RHT with ifosfamide and etoposide in patients with locally advanced sarcomas, with an overall response in 34% of patients [72].

Immunological implications

It is possible that the efficacy of hyperthermia does not relate solely to the killing of tumour cells by heat, but also to the induction of an immune response. Tumour cells from a Ewing's sarcoma cell line, exposed to a non-lethal temperature of 41.8°C, showed increased surface expression of hsp72. These cells were more susceptible to lysis by natural killer (NK)-like cells [73]. In contrast, cells derived from healthy individuals (fibroblasts, peripheral blood lymphocytes or monocytes) failed to show hsp72 surface expression, irrespective of the temperature to which they were exposed.

The possibility that γδ T-cells, a minor (approximately 5%) subpopulation of T-cells, recognise HSP-derived epitopes has previously been suggested in a number of diseases, and increased numbers of these cells have been found within tumours [74]. γδ T-cells exert cytotoxic function, but do not appear to recognise antigen in a MHC-restricted manner nor has their antigen specificity been established. Their repertoire appears more limited than αβ and, unlike αβ, they are essentially stationary, localised to areas such as the intraepithelial lining of the gut and in other epithelial layers, suggesting a possible role as the first line of defence, while an αβ T-cell response is activated [8]. Approximately 5% of γδ T-cell hybridomas, derived from neonatal mouse thymic cells, respond to mycobacterial hsp65, and these cells spontaneously produce IL-2 in the absence of stimuli, suggesting that they respond to a self-antigen [75, 76].

Therapeutic hyperthermia may thus potentiate the cytotoxic effects of γδ T-cells or NK cells by increasing HSP cell surface expression. In such a case, one would expect an increase in immune surveillance with a subsequent decrease in metastases. However, patients treated with combined therapies including hyperthermia appear as susceptible to recurrences of disease or distant metastases.

Thermotolerance, cross-resistance and multidrug resistance

Cells exposed to sublethal HS become resistant to temperatures otherwise lethal. This property, termed thermotolerance, has many implications for treatment of cells using hyperthermia [61]. A related phenomenon is cross resistance or multidrug resistance (MDR), whereby cells treated with one chemotherapeutic agent become resistant to this and other (not necessarily related) drugs. These phenomena may be partly due to the induction of members of the HSP which serve to protect the cells against further damage. In some human cancer cell lines, elevated levels of inducible hsp70 are linked with resistance to further treatment with doxorubicin [16]. These cells also show elevated levels of phosphorylated hsp27 isoforms [77].

Both thermotolerance and MDR are induced by heat and oppose the therapeutic effects of hyperthermia [61]. The protein product of the MDR1 gene, P-glycoprotein, is an energy-dependent efflux pump which prevents the accumulation of toxins within the cell. It is expressed in a tissue-specific manner in kidney, liver, brain and testis; tumours arising from these organs and other tissues express increased levels of P-glycoprotein [78]. The presence of functional HSE in the upstream promoter region of the MDR1 gene [79] may indicate that part of the drug resistance following hyperthermia and formerly attributed to HSPs, relates to upregulation of P-glycoprotein. Finally, drug resistance could also be due to a block in programmed cell death as is seen with deregulation of the proto-oncogene, *BCL-2* [29]. As the number of combined treatments increases, it is essential that no one treatment leads to the decreased success of another. Some studies suggest that hyperthermia regimens which are too close together promote resistance to subsequent treatment. How HSP and/or P-glycoprotein could exert this effect is not apparent as yet, but Mosser and Martin [58] demonstrated that cells which are thermotolerant are also resistant to apoptosis.

Resistance to chemotherapeutic drugs is not observed with all drugs, but is obvious with some. For example, amphotericin B, normally used as an antifungal agent, affects the plasma membrane and becomes cytotoxic at temperatures above 42°C. Cells which are resistant to amphotericin B are also heat-resistant, and it has been suggested that one mechanism by which thermotolerance is induced is via change(s) in the plasma membrane [61].

CONCLUSIONS

The evidence presented here indicates that HSPs are involved in several aspects of cancer and cancer therapy.

At the cellular level, HSPs interact with a variety of proteins with both beneficial and deleterious effects. Stabilisation of mRNA of proto-oncogenes and the mutant forms of p53 protein may prove to be harmful by increasing the lifetime of these molecules beyond that which is helpful to the organism. However, the interaction of hsp70 with mutant p53 may prevent mutant-WT p53 complex formation and thus allow WT p53 to function unhindered.

The recognition of HSPs as tumour antigens, and possibly as a novel form of presenting molecules, highlights a therapeutic tool not previously recognised. In the future, this may represent a new direction for the development of cancer vaccines, based on the conserved nature of the HSPs and features of the peptides which they carry or present.

Overall, most past and current evidence from the literature suggests that hyperthermia and/or HSPs may have therapeutical effects in cancer, clinically beneficial effects being particularly

important when combining hyperthermia with other anticancer agents. However, due to thermotolerance and multidrug resistance, which can jeopardise the outcome of therapy, hyperthermia must only be used under controlled regimens.

Further studies, both at the cell biology and at the clinical level, are required to determine the precise role of hyperthermia in anticancer therapeutic strategies.

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