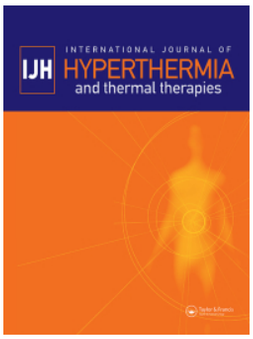

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How is the immune response affected by hyperthermia and heat shock proteins?

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Abstract
There is growing body of evidence linking the cellular response to heat stress with the response of the immune system to cancer. The anti-tumor immune response can be markedly enhanced by treatment with hyperthermia particularly in the fever range. In addition, the heat shock proteins (hsp) which are produced in abundant quantities in cells exposed to heat are potent immune modulators and can lead to stimulation of both the innate and adaptive immune responses to tumors. Immunostimulation by hyperthermia involves both direct effects of heat on the behavior of immune cells as well as indirect effects mediated through hsp release. In addition, the hsp can be deployed as components of antitumor vaccines in protocols that do not include hyperthermia. Understanding these process may permit the effective deployment of hyperthermia and hsp based vaccines in tumor treatment.

Keywords: *Heat shock protein, peptide complex, extracellular hyperthermia, tumor immune response*

Introduction

Hsp70 and other molecular chaperones are finding increasing use in tumour immunotherapy. Hsp70 has been employed as an adjuvant in combination with other treatments to activate APC and break tolerance to tumour-associated antigens or as a carrier protein to deliver tumour antigens to MHC class I molecules on APC [1]. The antigens chaperoned by hsp70 in vaccines can be individualized to patients' tumours or common to many tumour types. The hsp70 family is intrinsic to cellular life, permitting proteins to function within the tightly crowded milieu of the cell [2]. Hsp70 is increased in many types of cancer, even under non-stress conditions, protecting emerging cancer cells from the apoptosis that

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accompanies many steps in transformation, but also creating an opportunity for immune attack [2].

Role of hsp70 family members in antigen processing and capture

Hsp70 becomes associated with tumour antigens during antigen processing when damaged proteins are degraded through the ubiquitin-proteasome pathway to small peptides, a fraction of which are used for immune surveillance. Such peptides then become associated with major histocompatibility class I (MHC I) protein complexes where they are subject to surveillance by cytotoxic, CD8+ lymphocytes. Hsp70 family members bind peptides released into the cytoplasm from the proteasome and may be intermediates in transport to the MHC class I. This is largely inferred from the fact that hsp70 extracted from tumours can cross-present tumour antigens to antigen processing cells (APC) which are then recognized by specific clones of cytotoxic $CD8^+$ lymphocytes. It is not clear if hsp70 has selectivity for specific tumour antigens. Although some studies indicate a similar peptide binding preference for MHC I and hsp70, suggesting roles for hydrophobic and basic amino acids, substrate preferences in hsp70 *in vivo* are determined largely by J-domain co-chaperones that localize hsp70 to target molecules. Future investigation will be required to determine the relative selectivity of hsp70 for tumour antigens.

Escape of hsp70 proteins from the cytoplasm and release into the external milieu

The findings that hsp70 is released into the blood stream and stimulates production of anti-hsp70 antibodies was the first indication of extra-cellular hsp70 [3]. Circulating hsp70 is derived from dying cells and/or from hsp70 actively released from intact cells [3]. The terminal stages of necrosis appear to favour antigen uptake by hsp70, as intra-cellular ATP levels decline and peptides become locked onto the ADP-associated hsp70 [4]. Indeed, hsp70 over-expression in the presence of slow necrotic death is an extremely potent approach to breaking tolerance and induction of specific immune destruction of tumours [5]. Hsp70 peptide complexes (hsp70.PC) released from the tumour constitute a 'danger signal' attracting and activating APC [6]. Such hsp70.PC is likely released along with other molecular chaperones with pro-immune activity and other danger signals such as high-mobility group protein-1 (HMG-b1) [6]. Hsp70.PC are, however, not merely danger signals but carry tumour-associated antigens, which mediate to adaptive immunity. Indeed, injection of hsp70.PC or multi-chaperone based vaccines into tumour bearing hosts may mimic these effects [1].

Binding of hsp70.PC to the cell surface of antigen presenting cells

Hsp70.PC released from tumour cells becomes diluted on entering the interstitial fluid and APC response to low concentrations of hsp70.PC implies the existence of high-affinity receptors. Hsp70.PC have at least two activating effects on APC: (i) induction of innate immunity [7] and (ii) induction of the adaptive immunity through the transport of peptide antigens into APC, delivery to MHC class I and activation of cytotoxic T cells [1]. Four main candidate receptors have been suggested, including: (i) the CDI4/TLR 2/4 complex, (ii) the CD91 receptor, (iii) CD4O and (iv) scavenger receptors—most notably lectin-like receptor for oxidized low-density lipoprotein (LOX-1); reviewed in Theriault et al. [8]. However, recent studies comparing hsp70 binding to each of these receptors suggest that the

chaperone binds with high affinity to LOX-1 but not to the other three receptor classes, although the existence of additional receptors seems almost certain [8].

Internalization of HSP70.PC by APC, activation of transmembrane signalling cascades and re-presentation of peptides to cell surface MHC molecules

Hsp70 can initiate a potent innate immune response involving APC maturation [5]. Dendritic cell (DC) maturation involves expression of co-stimulatory CD40, OX40L, B7.1 and B7.2 molecules on the cell surface (needed for effective interaction with CD8+ T lymphocytes) [9]. CD40 has been shown to play a key role in the immune effects of hsp70 [7]. It seems likely that that hsp70-induced DC maturation involves CD40 and/or CD40L up-regulation and probably requires activation of the transcription factor NF-kB. Most of the genes involved in DC maturation require activation of NF-kB, suggesting that receptors involved in DC maturation by hsp70 cause activation of NF-kB [9]. Some intriguing studies show that extra-cellular hsp70 induces NF-kB through the activation of the CD14/TLR signalling pathway in a CD14-dependent manner in APC. However, this effect seems indirect since there is no evidence for high affinity binding of extra-cellular hsp70 to TLR 2, TLR 4 or CD14 [8]. In addition, some skepticism has been directed at the role of CD14 or TLR due to the potential of hsp70 contamination by endotoxin in these effects. Other potential hsp70 receptors, LOX-1 in particular, activate NF-kB and mediate induction of CD40 expression, suggesting a potential role for LOX-1 in APC maturation by hsp70.

A receptor-mediated uptake mechanism appears to be involved in antigen cross-presentation by hsp. After binding to hsp70.PC, such a receptor would mediate internalization of complexes and delivery of the peptide cargo to MHC class I molecules. Receptor-mediated protein internalization requires specific motifs located in the cytoplasmic tails of receptors, which engage the internalization machinery. Following receptor-hsp70-peptide complex uptake, complexes traffic through a number of intra-cellular compartments, leading to peptide release into the cytoplasm and re-presentation on the APC surface after binding to MHC class I. So far, no definitive studies have addressed the pathways of peptide cross-presentation through hsp70. Nonetheless, the internalization pathway of the stress protein gp96, which has similar immunological properties to hsp70, has been studied [10]. Gp96.PC is rapidly internalized, after interacting with undefined cell surface receptor(s), into a pre-endosomal compartment prior to transfer to MHC class I and presentation on the cell surface [10].

Hyperthermia

Fever-range hyperthermia also functions as a biological adjuvant, activating APC and tumour immunity through a number of mechanisms which appear to include both hsp-dependent and -independent effects [11]. At higher temperatures, hyperthermia may function to boost hsp expression and release as well as inducing independent effects on immune cell activation. Combination of fever range hyperthermia with chaperone-based vaccines seems a highly promising approach.

Conclusion

Extra-cellular hsp70.PC are effective agents for breaking tolerance to tumour antigens and eliciting specific $CD8^+$ tumour specific immunity and are highly effective agents for tumour immunotherapy. The pro-immune effects of hsp70 reflect its ability to act as both APC

activating ligand and as a carrier protein to chaperone tumour antigens for re-presentation by APC. However, most of the molecular mechanisms underlying these effects are not completely understood. Further progress will require a clearer understanding of the role of hsp70 in antigen processing in tumour cells, mechanisms of release of hsp70.PC from tumours, receptor mediated uptake of HSP70.PC by APC and mechanisms of hsp70-mediated re-presentation of tumour antigens to immune effector cells. Understanding these processes may permit one to manipulate more effectively the use of hsp70 in tumour immunotherapy.

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