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Mini-review

The role of heat shock protein 70 (Hsp70) in radiation-induced immunomodulation

Gabriele Multhoff [a,b,*], Alan G. Pockley [c], Thomas E. Schmid [a], Daniela Schilling [a]

[a] Department of Radiation Oncology, Technische Universität München, Klinikum rechts der Isar, Munich, Germany
[b] Helmholtz Center Munich, German Research Center for Environmental Health, CCG – "Innate Immunity in Tumor Biology", Munich, Germany
[c] John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham, UK

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ABSTRACT

Despite enormous progress in radiation technologies (high precision image-guided irradiation, proton irradiation, heavy ion irradiation) and radiotherapeutic concepts (hypofractionated irradiation schemes), the clinical outcome of radiotherapy in locally advanced and metastasized tumors and in hypoxic tumors which are radiation-resistant remains unsatisfactory. Given their key influence on a number of biological and immunological parameters, this article considers the influence of irradiation-induced stress proteins on radiation-induced immunomodulation. Depending on its location, the major stress-inducible Heat shock protein 70 (Hsp70) has been found to fulfill multiple roles. On the one hand, increased intracellular Hsp70 levels have been found to play a key role in the recovery from stress such as radio(chemo)therapy, and on the other hand extracellular Hsp70 proteins are potent stimulators of the innate immune system and mediators of anti-tumor immunity. Furthermore, if loaded with tumor-derived peptides, members of the Heat Shock Protein 70 (HSP70) and 90 (HSP90) families can stimulate the adaptive immune system via antigen cross-presentation. An irradiation-induced enhancement of the selective expression of a membrane form of Hsp70 on the surface of tumor cells which can act as a recognition structure for activated NK cells might have significant clinical relevance, in that the outcome of irradiation therapy for advanced tumors could be improved by combining it with cell-based and other immunotherapies that target this membrane form of Hsp70.

Major stress-inducible Heat Shock Protein 70 (Hsp70): function and subcellular localization

Heat Shock Proteins (HSPs) were firstly described by Ferruccio Ritossa in 1962 [1] as a set of evolutionary highly conserved genes in *Drosophila melanogaster* that are activated upon heat stress. Six of the eight members of the HSP70 family predominantly reside in the cytosol, where they maintain protein homeostasis by supporting the folding, refolding, and assembly of nascent polypeptides, preventing protein aggregation, and assisting the transport of other proteins across membranes [2]. Apart from heat, the synthesis of different HSP family members is increased by a large variety of different stressors including chemo- and radiation therapy which cause the production of reactive oxygen species (ROS) and also during cell proliferation and differentiation. A comparison of two highly homologous members of the HSP70 family, the constitutive Hsc70 (Hsp73, HSPA8, Hsp70-8) and the major stress-inducible Hsp70 (Hsp72, HSPA1A, Hsp70-1), has revealed that, under physiological conditions, the constitutive Hsc70 is expressed at higher levels than Hsp70, whereas the synthesis of Hsp70 is more rapid and accumulates in different subcellular compartments after stress [3,4]. Furthermore, in contrast to normal cells, tumors frequently overexpress Hsp70 in the cytosol, present Hsp70 on their plasma membrane [5,6], and actively release Hsp70 [7–9]. Elevated cytosolic levels of Hsp70 and Hsp27 have been found to mediate protection against apoptosis, promote malignant transformation, senescence and metastatic spread [10–15], whereas extracellular, tumor-derived HSPs are considered to act as danger signals [16] that can elicit anti-tumor immune responses [13,17–19]. A summary of the key actions of Hsp70 depending on their localization is shown in Table 1.

Membrane localization of Hsp70 on tumor cells is enabled by tumor-specific lipid components. Under physiological conditions Hsp70 co-localizes with the lipid raft glycolipid

Abbreviations: APC(s), antigen-presenting cell(s); ATP, adenosine triphosphate; Ca, calcium; Gb3, globoyltriaosylceramide; Grp, glucose-regulated protein; Gy, gray; HSP, Heat Shock Protein (refers to the family of Heat Shock Proteins); Hsp, Heat shock protein (refers to a specific member of the family); IFNγ, interferon gamma; mAb, monoclonal antibody; MHC, major histocompatibility molecules; NK cell, natural killer cell; NSCLC, non-small lung cell cancer; PS, phosphatidylserine; Treg cell, regulatory T cell.

* Corresponding author. Tel.: +89 4140 4514; fax: +89 4140 4299.
E-mail address: gabriele.multhoff@lrz.tu-muenchen.de (G. Multhoff).

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Table 1
Key actions of Heat shock protein 70 (Hsp70).

Localization	Function	References
Cytosolic	Binds to polypeptides in an ATP dependent manner	[20] [21]
	Prevents aggregation of unfolded peptides and transports proteins	[18]
	Regulates intercellular signaling	
	Mediates antigen cross-presentation	
Membrane-bound	Acts as a tumor-specific recognition structure for activated NK cells on plasma membranes	[22] [10]
	Mediates anti-apoptotic functions on lysosomal membranes	
Extracellular free and lipid-bound	Induces inflammatory and anti-inflammatory responses	[23] [24] [25] [26]

globoyltriaosylceramide (Gb3) [27], whereas Hsp70 is predominantly associated with phosphatidylserine (PS) outside of lipid rafts after stress. In non-stressed cells, the ATP-dependent aminophospholipid translocase [28] enables the exclusive localization of PS to the inner side of the plasma membrane. However, PS translocates from the inner to the outer plasma membrane leaflet via an activation of the ATP and Ca^{2+} dependent phospholipid scramblase after stress and thus provides an early marker of apoptosis [29]. Although the role of PS in the outer membrane leaflet is still not completely clear, it is assumed that oxidatively modified PS provides a phagocytic "eat-me" signal [30]. However, PS positivity does not necessarily mean that cells are no longer viable, as Hsp70/PS membrane-positive tumor cells are viable and can be grown in cell culture [31,32]. Furthermore, viable T cells also have been found to present PS on the outer leaflet following activation [33].

HSPs as targets for adaptive and innate immune responses

The potential of using members of families (particularly the 70 kDa and 90 kDa families) as potential chaperones for immunogenic peptides in the context of cancer immunotherapeutics has been considered by the group of Srivastava for many years [18]. Although the clinical success of this approach has been variable, the use of tumor-derived gp96 has been introduced into the clinical setting (Prophage Series of vaccines from Agenus: www.agenus.com/science/prophage.php). These studies were based in the concept that isolated from tumor tissue chaperoned tumor-specific peptides and, on administration, could induce the generation of peptide-specific effector cells. The specificity of the immunity that was generated was reported as being toward the antigenic peptides that were carried by the HSP, rather than the HSP itself. It was therefore proposed that this avoided potential cross reactivity with other tissues expressing the relevant. Some subgroups responded very well, whereas others have not profited from the therapy. Potential problems with this approach involved the limited amount of tissue from which the HSP (and therefore the vaccine) could be generated. Notwithstanding this, a number of clinical trials evaluating the clinical potential approach have been performed, and some success has been obtained [34]. As with all such studies, the translation of such therapies has been slow due to the fact that initial clinical trials are usually performed in patients with advanced disease.

Furthermore, the search for tumor-specific targets which are located intracellularly in normal cells, but are expressed on the cell surface of tumor cells and can be recognized by immune competent effector cells, has resulted in the identification of such as Hsp70, Hsp90, or Grp78 [35]. Herein, we concentrate on the major stress-inducible Hsp70 which is exclusively expressed on the cell surface of tumor cells, but not normal cells [5]. Ionizing irradiation, even at sublethal doses (below 5 Gy), induces the synthesis of Hsp70 in the cytosol of normal and tumor cells [36]. However, due to differences in the lipid composition of the plasma membrane only tumor cells have the capacity to present Hsp70 on their cell surface [27]. Irradiation as well as other stress factors such as heat, chemotherapeutic agents, Hsp90 inhibitors, amino acid analogues, glucose deprivation, hypoxia and reoxygenation, and drugs [37] further increases the cell surface density of Hsp70 on tumor cells. The cell surface bound form of Hsp70 on viable tumor cells is detectable using a mouse monoclonal antibody (cmHsp70.1, patent multimmune GmbH) [38], but not by other commercially available Hsp70-specific antibodies. The cmHsp70.1 monoclonal antibody (mAb) antibody detects the conformation of Hsp70 in the plasma membrane of tumor cells and does not cross-react with the highly homologous Hsc70.

Low dose irradiation up-regulates a number of immunologically-relevant antigens, such as major histocompatibility molecules (MHC), tumor-associated antigens (Carcinoembryonic Antigen (CEA)), mucin 1 [39], as well as the expression of the Intercellular Adhesion Molecule-1 (ICAM-1) on endothelial cells [40] and the apoptosis inducer Fas (CD95), all of which have immunomodulatory properties [41,42]. In addition to the direct killing of cancer cells by ionizing irradiation which is mediated by DNA damage can also induce non-targeted abscopal or bystander effects that can elicit the stimulation of T and NK cell mediated immune responses [42]. Most of these antigens are also relevant for the induction and regulation of T cell mediated immune responses and the generation of protective anti-tumor immunity [43]. T cells can be roughly grouped into $CD4^+$ T helper cells that recognize antigenic determinants presented by MHC class II molecules on antigen presenting cells (APCs), and $CD8^+$ cytotoxic T cells which recognize their target antigenic peptides that are presented in the context of MHC class I molecules [44]. $CD4^+$ T helper cells exist in different epigenetic states such as Th1, Th2, Th17, fork-head box 3 ($Foxp3^+$) T regulatory cells, T follicular helper, Th9, and Th22 cells [45] that are determining their different functions. Th17 cells show a high plasticity and thus can acquire pro-inflammatory characteristics of Th1 cells. T cells recognize MHC–peptide complexes via clonal T cell receptors which exhibit an enormous heterogeneity which is generated by variable diversity joining gene recombination and crossover events. T cell mediated immune responses, and the memory which they display, play a crucial role in immunosurveillance of cancer. However, effective anti-cancer immune responses can be blocked or down-regulated by regulatory T (Treg) cell populations which can be characterized, in part at least, on the basis of their expression of FoxP3 transcription factor, a high constitutive cell surface expression of the IL-2 receptor alpha chain (CD25), expression of the glucocorticoid induced TNF-receptor related protein GITR and the cytotoxic T lymphocyte associated antigen 4 (CTLA-4), as well as low levels of the IL-7 receptor (CD127) in humans [46]. Another level of control of anti-cancer immunity – so called "checkpoint inhibitors" – is also currently attracting a lot of attention. Under normal circumstances, immune checkpoints are important for maintaining self-tolerance by preventing autoimmunity and protecting the tissue from damage when the immune system is activated. However, it is now apparent that the expression of immune checkpoint proteins can be used to establish resistance mechanisms by tumor cells, thus allowing progressive tumor growth [47]. Attention has therefore turned to the potential therapeutic value of antibodies that target CTLA-4 and the programmed cell death protein 1/programmed cell death protein 1 ligand (PD1/PD-L1) to block such interactions and inhibit this protective response [48–50]. Immune checkpoint blockade is believed to have enormous therapeutic potential and integrative immunotherapies that incorporate immune checkpoint blockade should result in durable clinical responses and increased cure rates [51].

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