Published in final edited form as:

Expert Rev Vaccines. 2013 June ; 12(6): 581–583. doi:10.1586/erv.13.47.

Integrating a “danger” signal into molecular chaperoning to improve vaccination against cancer

Xiaofei Yu^{1,2,3}, John R. Subject⁴, and Xiang-Yang Wang^{1,2,3}

¹Department of Human and Molecular Genetics, Virginia Commonwealth University School of Medicine, Richmond, VA23298, USA

²VCU Institute of Molecular Medicine, Virginia Commonwealth University School of Medicine, Richmond, VA23298, USA

³VCU Massey Cancer Center, Virginia Commonwealth University School of Medicine, Richmond, VA23298, USA

⁴Department of Cellular Stress Biology, Roswell Park Cancer Institute, Buffalo, NY14263, USA

Keywords

molecular chaperone; dendritic cell; Grp170; flagellin; vaccine; antitumor immunity

Molecular chaperones are essential for maintaining cellular functions by assisting protein folding and translocation, or by preventing protein misfolding and aggregation [1]. Studies over the last two decades support the concept of intracellular chaperone molecules as carriers of the antigenic repertoire of cancer cells which is attributed to their intrinsic property to interact with polypeptide chains [2]. Autologous tumor-derived, chaperone-peptide complex preparations, therefore, provide an innovative immunotherapeutic approach to treatment of cancers. However, only limited improvement in the clinical outcome has been achieved using this approach [3]. Recombinant chaperone complex vaccines, which are built on the superior client or antigen-holding capability of large stress proteins (110 kDa heat shock protein, Hsp110 and 170 kDa glucose-regulated protein, Grp170), present an alternative vaccine strategy that effectively targets defined tumor antigens. Engineering a modified immunochaperone that is able to concurrently promote antigen cross-presentation and innate immune responses may lead to development of a new generation of chaperone-based vaccine therapy with improved clinical feasibility and vaccine efficacy.

Mechanisms underlying chaperone-based vaccine therapies

During the past decade much has been learned regarding the molecular basis for chaperone-mediated immune regulation that permits their use as vaccine adjuvants. A key feature is their ability to efficiently direct associated polypeptides into the endogenous antigen presentation pathway for cross-presentation by professional antigen presenting cells (APCs), such as dendritic cells (DCs). It has been well documented that chaperone interaction with various receptors on APCs is critical for enhanced internalization and cross-presentation of antigens carried by chaperone molecules. Several chaperone-binding structures on APCs, such as CD91 and scavenger receptors, including SRA, LOX-1, and SREC, have been

Correspondence: Xiang-Yang Wang, Department of Human & Molecular Genetics, PO Box 980033, Virginia Commonwealth University School of Medicine, Richmond, VA 23298. Tel: (804) 628-2679; xywang@vcu.edu.

All authors disclose no financial conflict of interest

described [4]. Unexpectedly, our studies using SRA knockout mice revealed that SRA acts as an immunosuppressor capable of attenuating an antitumor immune responses generated by chaperone-based vaccines [5,6]. It is likely that the different receptors may result in distinct immune outcomes upon binding to their ligands (e.g., chaperones). To selectively target chaperone-associated antigen to the receptors truly responsible for chaperone-facilitate T cell cross-priming, the exact activity of individual receptors in the context of chaperone-based vaccination needs to be determined using both *in vitro* and *in vivo* systems.

Independently from their antigen shuttling and presentation functions, chaperone molecules have been shown to promote the phenotypic and functional maturation of APCs (e.g., DCs and macrophages) by upregulating costimulatory molecules and inducing secretion of cytokines or chemokines. This highlights the potential of extracellular chaperones in bridging innate and adaptive immunity. While there is convincing evidence supporting the intrinsic immunostimulatory properties of chaperones [7], it should be noted that these molecules do not stimulate an innate immune response as strongly as pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides.

Autologous tumor-derived, chaperone-peptide complex vaccines

Due to their inherent chaperoning functions, certain chaperone molecules extracted from cancer cells can carry a tumor-specific, immunogenic peptide fingerprint [8]. This offers personalized cancer vaccine therapy and bypasses the need for the identification of tumor-associated antigens. Autologous tumor-derived, chaperone-peptide complexes have been extensively studied over the last two decades and have been proven to be effective in treating a number of malignant diseases in preclinical cancer models. Although the results from multiple phase I/II clinical trials of Gp96/Grp94-peptide complex (also known as vitespen, formerly HSPPC-96) were promising, the phase III trial failed to demonstrate overall survival benefits [3]. Introspective analysis revealed that vitespen treatment has benefited only subsets of early stage melanoma and kidney cancer patients, which also appeared to depend on the doses of vaccine delivered to these patients. Clinical trials with Gp96/Grp94-based vaccine vitespen in Glioblastoma patients are ongoing (NCT00293423, NCT00905060). Another related approach to generating chaperone-based autologous vaccines utilizes chaperone-rich lysates (CRCLs) [9], which are also being clinically tested as part of an immunotherapy regimen. In addition, Hsp70-peptide complex as an autologous vaccine can also be prepared from tumor-DC fusion cells that allow optimization of antigen processing and peptide loading [10].

Recombinant large chaperone-protein antigen complex vaccine

The clinical applications of autologous chaperone-peptide vaccines were hampered by the limited access to human tumor specimen and a complicated procedure for vaccine preparation. As a result, many patients were excluded from clinical trials due to insufficient quantity of vaccines [11]. Taking advantage of exceptional client or antigen-holding capability of large chaperones, Hsp110 and Grp170, we have developed a recombinant chaperone-protein complex vaccine, which is generated by complexing defined tumor protein antigen to these molecules under heat shock conditions *in vitro* [12]. These large chaperones are highly efficient in promoting cross-presentation of associated protein antigen and eliciting a robust cytotoxic T lymphocyte (CTL) response to tumors [12–15]. The ancient chaperoning function has been shown to be a major factor that determines the effective T cell cross-priming activity of these large chaperones [15]. Although the reconstituted chaperone-protein complex vaccine may lack the personalized nature of autologous chaperone-peptide vaccine, the protein antigen that contains a large reservoir of potential peptides can potentially stimulate polyepitope-directed T and B cells. Importantly,

this synthetic platform can be used to develop a multivalent vaccine targeting many different antigen targets [16]. As an “off-the-shelf” product that can be manufactured in large quantities, this vaccine may be used as adjuvant therapy for patients with completely resected disease or those at high risk for recurrence of cancer. A phase I clinical trial of recombinant Hsp110-gp100 chaperone vaccine for treatment of human melanoma is underway at the Roswell Park Cancer Institute in collaboration with the National Cancer Institute.

Incorporating “danger” signal into chaperoning-based vaccine regimen

Upregulation of co-stimulatory molecules and production of inflammatory cytokines during the activation of DCs are crucial for effective priming of T cells reactive with tumor antigens. It is well established that engaging the pathogen-sensing signaling pathways in APCs with PAMPs can potentiate antigen presentation and adaptive immunity. Emerging evidence shows that chaperones interaction with PAMPs synergistically stimulates an innate immune response [17,18]. Our recent study demonstrates that Grp170 binds highly efficiently to CpG oligodeoxynucleotides (CpG ODN), a agonist for toll-like receptor 9 and amplifies innate immunity that confers protection from *Listeria monocytogenes* [18]. Although earlier studies support the notion that extracellular chaperones possess direct immunostimulatory activity, the innate immune response generated by these molecules of mammalian origin may not be sufficient to functionally activate APCs that have captured antigens. It is conceivable that incorporation of a pathogen-associated “danger” signal into the recombinant chaperone vaccine regimen would lead to improved antitumor efficacy.

We recently constructed a chimeric chaperone by strategically fusing the flagellin-derived, NF- κ B-stimulating sequence to Grp170 [19]. This multifunctional molecule, termed flagrp170, has two distinct features that are essential for chaperoning-based vaccine therapy: promoting cross-presentation of associated tumor antigens and concurrently augmenting functional activation of DCs *via* engaging the NF- κ B signaling pathway [20]. Introducing this chimeric chaperone into DCs strongly activates them as indicated by substantial elevation of CD40 and CD86, as well as production of IL-12. The study of this chimeric immunochaperone was carried out in the setting of *in situ* vaccination by delivering a Flagrp170-encoding adenovirus [19]. Intratumoral administration of flagrp170 preferentially induces the Th1 polarization of the tumor microenvironment (TME), with characteristics of high levels of cytokine IL-12 and IFN- γ , increased recruitment of CD8⁺ and natural killer cells to the tumor sites. The systemic antitumor immunity leads to profound inhibition of both treated tumors and distant metastases in several preclinical cancer models. Although flagellin and flagrp170 induce similar levels of IL-12 in the TME, the flagrp170 is much more efficient than flagellin in provoking an effective CTL response to tumors, reinforcing a pivotal role of chaperoning by Grp170 in the stimulation of adaptive arm of the immune system. Our results are consistent with the view that coupling tumor antigens and an immunostimulating ‘danger’ signal into the same vaccine delivery cargo is crucial for optimal antigen cross-presentation by DCs. This modified large chaperone represents a novel immune modulator that can be exploited to overcome the immunosuppressive mechanism in the TME for improved treatment outcomes. A recombinant large chaperone-protein complex vaccine based on this chimeric chaperone is currently under investigation.

Future opportunities for chaperone-based vaccines

Extensive studies over the last two decades have demonstrated that molecular chaperoning represents an innovative platform for development of safe and effective vaccine therapies to promote the immune control of cancers. A better understanding of the mechanism of chaperone action in immune modulation is expected to provide new opportunities for

improving and optimizing chaperone-based cancer immunotherapy. Given their highly efficient capability in promoting antigen cross-presentation, integrating a strong “danger” signal or PAMP that further stimulates the innate arm of an immune response into autologous or recombinant chaperone vaccines should lead to enhanced therapeutic potency. With their proven safety profile, it is feasible to test chaperone-based vaccines either in adjuvant setting or in conjunction with conventional treatment modalities (e.g., radiotherapy, chemotherapy) in clinical trials. To overcome the immunoregulatory processes involved in maintenance of host immune homeostasis and/or tumor-induced immunosuppressive mechanisms that dampen the effectiveness of vaccines or immunotherapy, other approaches targeting immune checkpoint molecules or immunosuppressors (e.g., blockade of CTLA-4 or Treg) should also be the focus of the future research on chaperoning-based vaccines.

Acknowledgments

The work was supported in part by NIH research Grants (CA129111, CA154708, and CA099326), DOD/CDMRP W81XWH-10-PCRP-SIDA, and Harrison Scholarship.

References

1. Lindquist S, Craig EA. The heat-shock proteins. *Annu Rev Genet.* 1988; 22:631–677. [PubMed: 2853609]
2. Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. *Annu Rev Immunol.* 2002; 20:395–425. [PubMed: 11861608]
3. Srivastava PK, Callahan MK, Mauri MM. Treating human cancers with heat shock protein-peptide complexes: the road ahead. *Expert Opin Biol Ther.* 2009; 9(2):179–186. [PubMed: 19236248]
4. Calderwood SK, Theriault J, Gray PJ, Gong J. Cell surface receptors for molecular chaperones. *Methods.* 2007; 43(3):199–206. [PubMed: 17920516]
5. Wang XY, Facciponte J, Chen X, Subjeck JR, Repasky EA. Scavenger receptor-A negatively regulates antitumor immunity. *Cancer Res.* 2007; 67(10):4996–5002. [PubMed: 17510431]
6. Qian J, Yi H, Guo C, et al. CD204 suppresses large heat shock protein-facilitated priming of tumor antigen gp100-specific T cells and chaperone vaccine activity against mouse melanoma. *J Immunol.* 2011; 187(6):2905–2914. [PubMed: 21832164]
7. Henderson B, Calderwood SK, Coates AR, et al. Caught with their PAMPs down? The extracellular signalling actions of molecular chaperones are not due to microbial contaminants. *Cell Stress Chaperones.* 2010; 15(2):123–141. [PubMed: 19731087]
8. Murshid A, Gong J, Calderwood SK. Heat-shock proteins in cancer vaccines: agents of antigen cross-presentation. *Expert Rev Vaccines.* 2008; 7(7):1019–1030. [PubMed: 18767951]
9. Graner M, Raymond A, Akporiaye E, Katsanis E. Tumor-derived multiple chaperone enrichment by free-solution isoelectric focusing yields potent antitumor vaccines. *Cancer Immunol Immunother.* 2000; 49(9):476–484. [PubMed: 11092614]
10. Enomoto Y, Bharti A, Khaleque AA, et al. Enhanced immunogenicity of heat shock protein 70 peptide complexes from dendritic cell-tumor fusion cells. *J Immunol.* 2006; 177(9):5946–5955. [PubMed: 17056519]
11. Oki Y, Younes A. Heat shock protein-based cancer vaccines. *Expert Rev Vaccines.* 2004; 3(4): 403–411. [PubMed: 15270645]
12. Wang XY, Chen X, Manjili MH, Repasky E, Henderson R, Subjeck JR. Targeted immunotherapy using reconstituted chaperone complexes of heat shock protein 110 and melanoma-associated antigen gp100. *Cancer Res.* 2003; 63(10):2553–2560. [PubMed: 12750279]
13. Manjili MH, Henderson R, Wang XY, et al. Development of a recombinant HSP110-HER-2/neu vaccine using the chaperoning properties of HSP110. *Cancer Res.* 2002; 62(6):1737–1742. [PubMed: 11912148]

14. Manjili MH, Wang XY, Chen X, et al. HSP110-HER2/neu chaperone complex vaccine induces protective immunity against spontaneous mammary tumors in HER-2/neu transgenic mice. *J Immunol.* 2003; 171(8):4054–4061. [PubMed: 14530326]
15. Park JE, Facciponte J, Chen X, et al. Chaperoning function of stress protein grp170, a member of the hsp70 superfamily, is responsible for its immunoadjuvant activity. *Cancer Research.* 2006; 66(2):1161–1168. [PubMed: 16424054]
16. Wang XY, Sun X, Chen X, et al. Superior antitumor response induced by large stress protein chaperoned protein antigen compared with peptide antigen. *J Immunol.* 2010; 184(11):6309–6319. [PubMed: 20439916]
17. Warger T, Hilf N, Rechtsteiner G, et al. Interaction of TLR2 and TLR4 Ligands with the N-terminal Domain of Gp96 Amplifies Innate and Adaptive Immune Responses. *Journal of Biological Chemistry.* 2006; 281(32):22545–22553. [PubMed: 16754684]
18. Zuo D, Yu X, Guo C, et al. Molecular chaperoning by glucose-regulated protein 170 in the extracellular milieu promotes macrophage-mediated pathogen sensing and innate immunity. *Faseb J.* 2012; 26(4):1493–1505. [PubMed: 22207611]
19. Yu X, Guo C, Yi H, et al. A Multifunctional Chimeric Chaperone Serves as a Novel Immune Modulator Inducing Therapeutic Antitumor Immunity. *Cancer Res.* 2013
20. Andreakos E, Williams RO, Wales J, Foxwell BM, Feldmann M. Activation of NF-kappaB by the intracellular expression of NF-kappaB-inducing kinase acts as a powerful vaccine adjuvant. *Proceedings of the National Academy of Sciences.* 2006; 103(39):14459–14464.