Heat Shock Proteins and Their Use as Anticancer Vaccines

Giorgio Parmiani,1 Alessandro Testori,2 Michele Maio,3 Chiara Castelli,1 Licia Rivoltini,1 Lorenzo Pilla,1 Filiberto Belli,1 Vincenzo Mazaferro,1 Jorgelina Coppa,1 Roberto Patuzzo,1 Mario R. Sertoli,4 Axel Hoos,5 Pramod K. Srivastava,6 and Mario Santinami1

1Istituto Nazionale Tumori and 2European Institute of Oncology, Milan, Italy; 3University of Siena, Siena, and 4Istituto Scientifico Tumori, Genoa, Italy; and 5Antigenics, Inc., New York, NY; and 6University of Connecticut, Farmington, Connecticut

INTRODUCTION

Despite the improvement in the outcome of anticancer therapy achieved during the last few years, several metastatic tumors remain resistant to therapy. This is particularly true for metastatic melanoma, renal and lung carcinoma, and, to a lesser extent, for colorectal carcinoma. For these tumors, several biological therapies have been proposed, including vaccination by a variety of approaches. Over the past decade, it has become increasingly apparent that the host immune system can recognize and destroy cancer cells, although this will only rarely translate into a significant clinical benefit (1). Consequently, antitumor therapies have focused on exploiting the immune response in an attempt to increase its strength and specificity to better control the disease.

Many tumor-associated antigens (TAAs) are now available in the form of single peptide epitopes restricted by known HLA class I or II alleles (see ref. 2). However, all of the TAAs used in clinical protocols belonged to the group of normal (i.e., self) peptides/proteins (e.g., gp100, tyrosinase, CEA) shown to be weakly immunogenic in vivo. This may be one important reason for the limited clinical response rate observed in these studies, in addition to mechanisms of immune evasion, which have been reported previously (3).

Thus, we believe that a new group of TAAs needs to be explored as for purposes of anticancer vaccine development, namely the individual (unique) TAAs that often result from mutations of a variety of proteins. In animal models, these TAAs enable rejection of tumors more potent than that obtained by shared TAAs (4).

Received 6/29/04; revised 8/27/04; accepted 9/2/04.

Grant support: Antigenics, Inc. (New York, NY).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Giorgio Parmiani, Unit of Immunotherapy of Human Tumors, Istituto Nazionale Tumori, Via Venezian 1, 20133 Milan, Italy. Phone: 39-0223902328; Fax: 39-0223902630; E-mail: giorgio.parmiani@istitutotumori.mi.it.

©2004 American Association for Cancer Research.

Perspective

Such individual antigens do exist also in human tumors (see ref. 2) and appear to be associated with long-term disease-free condition in the few patients that could be studied (5–7). However, with today’s technology, it still requires several months before novel TAAs are identified and characterized for the use in cancer vaccines. In addition, the heterogeneity of human cancers with regard to their molecular background and the resulting antigenic repertoire warrants a polyvalent approach to efficiently address larger numbers of patients within a given disease population. An alternative approach, which carries the promise to circumvent the above challenges, is offered by the use of tumor-derived heat shock protein-peptide complexes (HSPPCs) as cancer vaccines. In fact, Srivastava et al. (8) have shown that tumor-derived autologous heat shock proteins (HSPs) function as chaperones of TAA peptides, and the use of HSPPCs allows for immunization of the host against a large repertoire of individual TAAs. Moreover, mice immunized with heat shock proteins (HSPs) in a therapeutic setting often showed retarded progression of primary cancers and reduced metastatic load (9). In the following sections, we shall discuss how this principle has been applied for designing new clinical trials of cancer vaccines.

Preclinical Characterization of HSPs as Cancer Vaccines

The molecular chaperones HSP70, Grp94/gp96, calreticulin, and gp110 are well characterized within the family of HSPs. HSP70 has diversified functions, which include chaperoning protein into degradation pathways, binding of new synthesized amino acid chains on ribosomes, and maintaining translocation–competent folding of estrogen receptor and mitochondrial precursor proteins in the cytosol. As for the estrogen receptor resident Grp94/gp96, several authors showed that also this protein displays intrinsic polypeptide-binding activity (see ref. 10). Gp110 is related to HSP70 in structure and functions, but it appears more effective in binding and chaperoning full-length proteins (11).

However, not much is known on the structural requirements for peptide binding to HSPs because the primary sequences of very few peptides directly eluted from HSP70 and gp96 are available (12, 13). An important new feature of HSPs is that they bind specifically to antigen-presenting cells through toll-like receptors (TLRs), thus triggering the innate immunity signaling pathways (14, 15).

To investigate whether gp96 and HSP70 purified either from human tumors or in vitro cultured melanoma lines could indeed contain TAA peptides, we assessed the ability of tumor-derived gp96 and HSP70 to reconstitute the epitopes recognized by CD8+ T cells specific for melanoma or colorectal carcinoma antigens. For gp96, experiments showed that HLA-A*0201–restricted CD8+ T cells recognizing melanoma (e.g., Melan-A/ MART-1) or colorectal carcinoma (CEA and EpCAM) antigens were triggered to release IFN-γ by HLA-A*0201–matched an-
tigen-presenting cells pulsed with HSP96 purified from tumor cells expressing the relevant TAA (16).

Using a similar approach, we and others showed that also HSP70, purified from a human melanoma line, included peptides from Melan-A/MART-1, gp100, TRP-2, and tyrosinase (17, 18). Of note, not only HLA-A*0201 but also HLA-A*0301 and HLA-C*0801–restricted CTLs were specifically triggered by HSP70 derived from the autologous melanoma line 15392, thus indicating that different epitopes were chaperoned by HSP70 deriving from a single tumor (17).

Unfortunately, because T-cell clones specific for unique class I HLA-restricted melanoma TAAs were not available, the presence of peptides derived from these antigens inside the HSP70 chaperoned peptides could not be assessed, and therefore, the relation of shared versus unique class I HLA-restricted epitopes in human HSP70-chaperoned peptide repertoire is unknown. Altogether, more studies are necessary to prove that unique TAA peptides can bind different members of the HSP families.

Tumor-derived HSPPC-96 appeared to activate TAA-specific T cells and expand them in vivo. In fact, immunization with autologous tumor-derived HSPPC-96 induced a significant increase in the frequency of T lymphocytes recognizing Melan-A/MART-127–35, CEA571–579, and EpCAM263–271 in a subset of autologous tumor-derived HSPPCs have been shown to display another crucial immunologic function, i.e., the activation of innate immune responses by promoting the maturation of antigen-presenting cells in vitro and in vivo (21, 22) by binding specific TLRs (22, 23). Moreover, a 14-amin sequence of HSP70 has been identified that is specifically recognized by human natural killer cells (24).

This conclusion, however, has been criticized because of the possibility that (a) minute amounts of endotoxins may be responsible for this phenomenon despite the use of different methods aimed at eliminating or reducing such contaminants, or (b) HSPs themselves directly bind bioactive lipids such as lipopolysaccharide and transport it to the antigen-presenting cell receptor (25). Although it is almost impossible to exclude that undetectable amounts of endotoxins may contaminate or bind HSPs, several findings do not corroborate such an explanation for the antigen-presenting cell activation function of HSPs. In fact, in our ex vivo studies, HSP-96 obtained by the same procedure from histologically different or antigenically unrelated human tumors were used as negative controls, and antigen-presenting cells pulsed with such preparations were not recognized by TAA-specific T-cell clones (16, 17). Moreover, recent studies in animal models indicate that HSP can be co-administered with endotoxin in vivo and CD8+ T cells and activate them both in vitro and in vivo.

HSPPC's have been shown to display another crucial immunologic function, i.e., the activation of innate immune responses by promoting the maturation of antigen-presenting cells in vitro and in vivo (21, 22) by binding specific TLRs (22, 23). Moreover, a 14-amin acid sequence of HSP70 has been identified that is specifically recognized by human natural killer cells (24).

This conclusion, however, has been criticized because of the possibility that (a) minute amounts of endotoxins may be responsible for this phenomenon despite the use of different methods aimed at eliminating or reducing such contaminants, or (b) HSPs themselves directly bind bioactive lipids such as lipopolysaccharide and transport it to the antigen-presenting cell receptor (25). Although it is almost impossible to exclude that undetectable amounts of endotoxins may contaminate or bind HSPs, several findings do not corroborate such an explanation for the antigen-presenting cell activation function of HSPs. In fact, in our ex vivo studies, HSP96 obtained by the same procedure from histologically different or antigenically unrelated human tumors were used as negative controls, and antigen-presenting cells pulsed with such preparations were not recognized by TAA-specific T-cell clones (16, 17). Moreover, recent studies in animal models indicate that HSPs can stimulate in vivo and CD8+ T-cell response through activation of dendritic cells, which is independent of lipopolysaccharide and occurs via the TLR4 (26). A separation between antigen delivery and dendritic cell stimulation in HSP70 mycobacterial binding domain has also been reported previously (27).

Thus, although endotoxin contaminants cannot be completely ruled out in the biological activity of HSPs, the role of these contaminants, if any, cannot explain the bulk of the important immunologic functions exerted by these proteins particularly in chaperoning TAA peptides.

Taken together, the above findings, along with the demonstration of antitumor activity of HSPPCs in animal models, provided the rationale for HSPPC-based vaccination in cancer patients.
HSPs and Their Use as Anticancer Vaccines

An antitumor immune response in these data suggest that HSPPC-96 vaccination not only generated a statistically significant survival advantage for both disease-free and overall survival at 48 months (Table 1). Although, because of the limited number of patients, a possible influence of other prognostic factors could not be assessed.

Another phase II study was done by using HSPPC-96 to vaccinate patients after resection of liver metastases of colorectal carcinoma with a schedule similar to that used for melanoma (19). Patients with colorectal carcinoma who had developed liver metastases after initial treatment of their primary tumors underwent complete surgical resection of their liver disease, and HSPPC-96 was prepared from liver lesions. Four to 6 weeks after surgery, patients were given HSPPC-96. The disease-free and overall survival now at a median follow-up of 48 months in 29 evaluable patients, who received at least four doses of HSPPC-96, were comparable with those reported by our and other groups for similar patients undergoing the same kind of surgery followed by different types of chemotherapeutic regimens. However, a different clinical outcome was reported between the group of patients showing an increased, class I HLA-restricted T-cell response to colorectal carcinoma cells (n = 17) via the enzyme-linked immunospot assay and those who did not show such a response (n = 12). Immune responders had a statistically significant survival advantage for both disease-free and overall survival at 48 months (Table 1). Although, because of the limited number of patients, a possible influence of other prognostic factors could not be excluded, these data suggest that HSPPC-96 vaccination not only generated an antitumor immune response in >50% of the subjects but that it may also have contributed to improve patient’s clinical outcome.

Other Current Clinical Trials with HSPPCs

To date, several other clinical studies have been completed or are ongoing investigating the clinical benefit of autologous cancer-derived HSPPC-96 for patients in either the adjuvant or metastatic disease settings (see ref. 29). In fact, there are currently >150 medical centers worldwide enrolling patients in randomized, controlled phase III clinical trials testing this vaccine for the treatment of renal cancer and melanoma. In addition, autologous HSPPCs are being tested in phase I and II trials of chronic myelogenous leukemia, non-Hodgkin’s lymphoma, non–small-cell lung, pancreatic, and gastric cancers (Table 2).

In an attempt to investigate options to increase the immunogenicity of HSPPC-96, we started a new trial in metastatic melanoma patients (30) by administering HSPPC-96 together with the granulocyte macrophage colony-stimulating factor given at the site of vaccination for 3 subsequent days (−1, 1, +1, 1 being the day of vaccine) and followed by two s.c. injections of $3 \times 10^6$ IU of IFN-α2b to up-regulate the expression of class I HLA often decreased in metastatic melanoma cells (3). This regimen was given four times weekly, and after 4 weeks of rest, a second cycle of biweekly vaccinations was repeated. The immune response both in terms of frequency and intensity of induction of anti-TAA T cells was not increased compared with the previous study carried out in similar melanoma patients. Table 3 summarizes the anti-melanoma T-cell response, which was found either significantly increased or de novo activated after vaccination with HSPPC-96. With the possible exception of the recent study of HSPPC-96 given with the granulocyte macrophage colony-stimulating factor, the frequency of subjects showing an immune response was reproducible (48 to 52%), a finding of interest if one considers that most of the patients, particularly those with melanoma, had a significant tumor burden.

One of the unsolved issues of vaccination is the identification of surrogate markers predictive for clinical response,

**Table 1** Immune and clinical response in HSPPC-96–vaccinated colorectal carcinoma patients

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>No. of patients</th>
<th>Overall survival</th>
<th>Disease-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune responders</td>
<td>17</td>
<td>53</td>
<td>41</td>
</tr>
<tr>
<td>Immune nonresponders</td>
<td>12</td>
<td>17</td>
<td>0</td>
</tr>
</tbody>
</table>

* Responses were evaluated at 48 months of follow-up.

**Table 2** Summary of clinical trials testing autologous HSPs in immunotherapy of cancer

<table>
<thead>
<tr>
<th>Clinical phase</th>
<th>Cancer type</th>
<th>Clinical stage</th>
<th>No. of patients treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II Renal cell carcinoma</td>
<td>IV, disease-free post nephrectomy at high risk for recurrence</td>
<td>Target 650, ongoing</td>
<td></td>
</tr>
<tr>
<td>I/II Renal cell carcinoma</td>
<td>IV</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>II Renal cell carcinoma</td>
<td>IV, untreated</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>I/II Melanoma</td>
<td>IV, failed other therapies</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>I/II Melanoma</td>
<td>Advanced stage III, stage IV</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>I/II Melanoma</td>
<td>Advanced</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>I/II Gastric cancer</td>
<td>Chronic phase</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>II Colorectal cancer</td>
<td>Stage IV completely resected</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>I/II Gastric cancer</td>
<td>Chronic phase</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>I/II CML</td>
<td>CML †</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>I CML</td>
<td>Chronic phase</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>I Multiple advanced cancers</td>
<td>IV, failed other therapies</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>I Pancreatic cancer</td>
<td>IV-III</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>I/II Renal cell carcinoma</td>
<td>Low grade</td>
<td>17, ongoing</td>
<td></td>
</tr>
<tr>
<td>I Non-Hodgkin’s lymphoma</td>
<td>IV-III</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>I Pancreatic cancer</td>
<td>Low grade</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>I/II Renal cell carcinoma</td>
<td>Low grade</td>
<td>17, ongoing</td>
<td></td>
</tr>
<tr>
<td>I Renal cell carcinoma</td>
<td>IV</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>II Renal cell carcinoma</td>
<td>IV</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>III Renal cell carcinoma</td>
<td>IV</td>
<td>72</td>
<td></td>
</tr>
</tbody>
</table>

* The vaccine is HSPPC-96.
† CML, chronic myelogenous leukemia; the vaccine is HSPPC-70.
which may be found in one or more quantitative ex vivo immunologic assays. However, an association between T-cell response and clinical outcome begins to emerge from some of the most recent vaccination trials (19, 20, 31, 32). In our trials with HSPPC-96, an association was found between class I HLA-restricted antitumor T-cell response and clinical outcome, although a higher number of clinically responding patients is necessary to corroborate these early findings (19, 20).

Safety has been carefully investigated in the studies of vaccination with HSPPCs because of the possibility that self-TAA contained in such preparations could potentially trigger autoimmune reactions. However, no significant laboratory or clinical signs of autoimmunity were reported in the clinical trials done, to date, in >800 patients treated. The safety profile of HSPPC-96 is mostly characterized by mild and transient side effects such as local reactions or low-grade fever. This allows a reasonably good quality of life during treatment.

Limitations of the HSP vaccination approach consist of the requirement of tumor tissue to be removed by surgery and the time necessary for vaccine manufacturing, which may delay or even prevent (in case of disease progression) the treatment. Strategies are being developed at the preclinical and clinical level to solve these problems even with the use of recombinant HSPs and gene manipulation of tumor cells (33, 34).

CONCLUSIONS

HSPs represent an interesting family of proteins that may function as in vivo chaperones of TAA epitopes carrying peptides, although such conclusion requires additional experimental evidence. However, it appears clear that at least some T-cell epitopes deriving from human TAA are associated with gp96 and potentially endowed with the ability to stimulate antitumor T cells. The alternative explanation that this may occur through the activation of innate immunity that will then favor the triggering of TAA-specific T cells (35) is difficult to rule out in vivo but can be excluded in most in vitro representation assays with TAA-specific T-cell clones. Thus, at the moment, we conclude that gp96 and HSP70 preparations obtained from human tumors do contain several of the known TAA peptides, which can be recognized by the patients’ immune system.

However, the HSPPC vaccination approach is based on the principle that HSPPCs can potentially bind the full repertoire of the TAA of a single tumor, including the individual strong antigens that make each tumor antigenically different from the other. By this mechanism, tumor-derived HSPPCs should provide vaccines tailored to the individual patient’s cancer.

ACKNOWLEDGMENTS

We thank the expert technical work of Agata Cova, Gloria Sovena, and Paola Squarcina and the editing assistance of Grazia Barp.

REFERENCES


Heat Shock Proteins and Their Use as Anticancer Vaccines

Giorgio Parmiani, Alessandro Testori, Michele Maio, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/10/24/8142

Cited articles
This article cites 32 articles, 21 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/10/24/8142.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/10/24/8142.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.