

# Hsp60 and Hsp10 as Antitumor Molecular Agents

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## KEY WORDS

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## ABSTRACT

The molecular chaperones Hsp60 and Hsp10 are, according to recent reports, involved in cancer development and progression. We, for instance, have found that their expression varies with distinctive patterns in different malignancies: they are overexpressed in colorectal, exocervical and prostate carcinogenesis, and colorectal cancer progression, but they are downregulated during bronchial carcinogenesis. There is also evidence showing that Hsp60 and Hsp10 can be used as therapeutic agents, for example in rheumatoid arthritis. In view of these findings we want now to call attention to the potential of Hsp60 and Hsp10 in cancer therapy.

There is increasing evidence showing that the molecular chaperones Hsp60 and Hsp10 can be used as therapeutic agents, for example in rheumatoid arthritis.<sup>1-3</sup> We study these chaperones that work as a rule in conjunction with one another,<sup>4,5</sup> and we have found that their expression varies with distinctive patterns in different malignancies. They are overexpressed in colorectal, exocervical and prostate carcinogenesis,<sup>6-9</sup> and colorectal cancer progression,<sup>10</sup> but are downregulated during bronchial carcinogenesis.<sup>11</sup> In view of these reports and findings we would like to call attention to the potential of Hsp60 and Hsp10 in cancer therapy.

Human Hsp60 and Hsp10 are coded by well characterized single genes, which should facilitate genetic manipulations. Moreover, the proteins have been studied in a variety of organisms, which means that abundant information on structure, function, and substrates is available to help in the selection of therapeutic targets and to predict therapeutic effects at the molecular level.

The promise of Hsps for cancer treatment became apparent when it was observed that Hsps purified from a given tumor elicited specific immunity against that particular tumor.<sup>12,13</sup> In addition, Hsps participate as immunomodulators in both innate and acquired immune responses as discussed below, suggesting possible clinical applications.

Hsps could be used to enhance antitumor immunity in at least two ways. One of these would involve potentiation of the immune response through administration of a poorly antigenic tumor peptide transported by an Hsp molecule as in a hapten-carrier complex, which would heighten the immunogenicity of the peptide. In this case the term "Hsp-based antitumor vaccine" would designate a compound formed by an Hsp molecule and a tumor peptide antigen.

Another way to apply Hsps in antitumor therapy would involve administration of Hsps (protein therapy) or *hsp* genes (gene therapy) to provide selected target cells with the necessary doses of chaperones. In this instance, the mechanism responsible for the antitumor effects of Hsps would involve immunoregulatory pathways that lead to vigorous immune responses and that need Hsps at one or more stages; initial administration of an antigen-Hsp complex would not be a requirement. However, the Hsp will interact with antigen intracellularly at some point during antigen processing and presentation. In this case, the term antitumor vaccine would designate a purified Hsp preparation or gene construct with antitumor potency but devoid of a tumor-antigen moiety.

Hsp-based antitumor therapy offer a promising alternative in cancer management as suggested by the fact that some Hsp-based vaccines have passed the animal experimentation stage and have been tested in clinical trials.<sup>14</sup> Structural and functional studies indicate that the immunogenicity of some tumor-derived Hsps is chiefly due to chaperone-associated antigenic tumor peptides.<sup>15</sup> The chaperone moiety increases the immunogenicity of the tumor peptide (itself a weak immunogen). The immune response elicited by an Hsp obtained from tumor cells and carrying tumor-derived antigen is specific,

and is directed against the tumor from which the Hsp was purified but not against an antigenically distinct tumor or against normal cells (absence of side effect due to autoimmunity).<sup>16</sup> Thus, these vaccines are tumor- and patient-specific.<sup>17</sup>

To the present, considerable attention has been directed to the antitumor effects of Hsc70, Hsp70, Hsp110, GRP94/gp96a and Hsp90.<sup>14</sup> We would like to propose that Hsp60 offers a promising alternative as an antitumor agent as well, and that in this context Hsp10 should also be considered because the typical functions of Hsp60 involve Hsp10.<sup>4</sup> Surely, one cannot rule out that either chaperone could act alone (without the other) in many cellular processes, including in antitumor pathways, but from what we know at this time, it seems justified to consider both of them acting as a team. Published data indicate that Hsp60 can serve as antigen carrier, and in addition it can per se, in the absence of antigen, act as immunomodulator, but the coparticipation of Hsp10 in these processes has not been excluded. Evidence of involvement of Hsp60 in immunological phenomena, some with regard to cancer, comes from various sources:

(1) human Hsp60 was found overexpressed or downregulated in tumors;<sup>6-11</sup>

(2) the chaperone would specifically interact with antigen-presenting cells (APC) and boost secretion of pro-inflammatory cytokines by macrophages and dendritic cells, promoting maturation of dendritic cells;<sup>18</sup>

(3) highly purified human Hsp60 triggered human T lymphocyte proliferation in vitro.<sup>19</sup> The response to Hsp60 seemingly was driven by the naive CD45RA+ RO- T cell subset or cord-blood cells. In contrast to CD45RA+ RO- (naive) and CD45RA- RO+ (memory) cells, T cell populations proliferated after stimulation with bacterial Hsp60 from *Mycobacterium leprae*, *Escherichia coli* or *Chlamydia trachomatis*;

(4) as human Hsp60 acts not only directly on antigen-presenting cells (APC) by inducing the secretion of cytokines but also on other leucocytes, the impact of Hsp60 on the antigen-specific activation of CD8+ T cells was studied.<sup>20</sup> It was found that Hsp60 enhances the activation of cytotoxic T cells through activated macrophages, thus acting as a molecular link between innate- and adaptive-immune systems;

(5) IL-12 was found to play a central role in Hsp60-mediated IFN $\gamma$  induction, which was dependent on costimulatory signals provided by B7-CD28 cells;<sup>21</sup>

(6) peripheral blood lymphocytes (PBL) produced Th1-associated cytokines, including IFN $\gamma$  and IL-12, spontaneously and in response to stimulation with exogenous HSP60-derived peptide;<sup>22</sup>

(7) human Hsp60 acts as a costimulator of the human regulatory T cells (T<sub>regs</sub>) CD4+CD25<sup>int</sup> and CD4+CD25<sup>hi</sup>;<sup>23</sup>

(8) Hsp60 inhibited IFN $\gamma$  and TNF $\alpha$  secretion and upregulated IL-10 secretion by activated CD4+ T cells.<sup>23</sup> Hsp60 could very well act as a costimulator of CD8+ lymphocytes, and Hsp60-treated CD4+ CD25+ T cells could regulate CD8+ T cell cytokine secretion. Hsp60 specifically affected cytokine secretion in the CD3+ and CD4+ populations, and indirectly in CD8+ T cells;

(9) soluble Hsp60 can directly activate T cells via TLR2 signaling to enhance Th2 response and Hsp60 can activate B cells by an innate signaling pathway;<sup>24</sup> The human Hsp60, but not the related *Escherichia coli* GroEL or *Mycobacterium leprae* Hsp65, induced naive mouse B cells to proliferate and to secrete IL-10 and IL-6 and triggered the up-regulation of MHC class II and accessory molecules CD69, CD40, and also induced B7-2 expression;

(10) endocytosis of human Hsp60 by innate immune cells activated the stress-activated protein kinases p38 and JNK1/2 (JNK), the mitogen-activated protein kinases ERK1/2, and the IKK kinase (IKK).<sup>25</sup> Activation of JNK and IKK proceeded via the Toll/IL-1 receptor signaling pathway, involving MyD88 and TRAF6;

(11) Hsp60 expressed on the surface of eukaryotic cell lines increased the activation of T cells in primary stimulation and promoted cytokine production by APCs;<sup>26</sup> and,

(12) endogenous Hsp60 released from dying cells in vivo, provides a danger signal of tissue damage to the immune system.<sup>27</sup> This signalling function, independent from association with antigen, would also serve as a powerful enhancer of the immune response.

The publications cited above on the direct and indirect immunostimulatory properties of Hsp60 constitute a minimal sample of what has been published on the matter. It is thus justified to consider this chaperone among the candidates for cancer immunotherapy and, because Hsp60 typically functions in association with Hsp10, the latter should not be ignored. In practice, it is also encouraging that the *hsp60* and *hsp10* genes are well characterized, and that Hsp60 can be recovered from low amounts of tumor tissue in useful quantities endowed with low toxicity and inherent antitumor properties.<sup>28</sup> There is no reason to believe that Hsp10 will not be as efficiently obtainable from tumor cells as Hsp60, allowing the therapeutic use of both chaperones simultaneously or in succession.

## References

- Santos-Junior RR, Sartori A, De Franco M, Filho OG, Coelho-Castelo AA, Bonato VL, Cabrera WH, Ibanez OM, Silva CL. Immunomodulation and protection induced by DNA-hsp65 vaccination in an animal model of arthritis. *Hum Gene Ther* 2006; 16:1338-45.
- Lehman TJ. Juvenile idiopathic arthritis and HSP60 vaccination: Selective downregulation? *Lancet* 2005; 366:9-10.
- Vanags D, Williams B, Johnson B, Hall S, Nash P, Taylor A, Weiss J, Feeney D. Therapeutic efficacy and safety of chaperonin 10 in patients with rheumatoid arthritis: A double-blind randomised trial. *Lancet* 2006; 368:855-63.
- Macario AJL, Conway de Macario E. Sick chaperones, cellular stress, and disease. *N Engl J Med* 2005; 353:1489-501.
- Czarnecka AM, Campanella C, Zummo G, Cappello F. Mitochondrial chaperones in cancer: From molecular biology to clinical diagnostics. *Cancer Biol Ther* 2006; 5:714-20.
- Cappello F, Bellafiore M, Palma A, Marciàno V, Martorana G, Belfiore P, Martorana A, Farina F, Zummo G, Bucchieri F. Expression of 60-kD heat shock protein increases during carcinogenesis in the uterine exocervix. *Pathobiology* 2002; 70:83-8.
- Cappello F, Bellafiore M, David S, Anzalone R, Zummo G. Ten kilodalton heat shock protein (HSP10) is overexpressed during carcinogenesis of large bowel and uterine exocervix. *Cancer Lett* 2003; 196:35-41.
- Cappello F, Rappa F, David S, Anzalone R, Zummo G. Immunohistochemical evaluation of PCNA, p53, HSP60, HSP10 and MUC-2 presence and expression in prostate carcinogenesis. *Anticancer Res* 2003; 23:1325-31.
- Cappello F, Bellafiore M, Palma A, David S, Marciàno V, Bartolotta T, Sciume C, Modica G, Farina F, Zummo G, Bucchieri F. 60kDa chaperonin (HSP60) is overexpressed during colorectal carcinogenesis. *Eur J Histochem* 2003; 47:105-10.
- Cappello F, David S, Rappa F, Bucchieri F, Marasa L, Bartolotta TE, Farina F, Zummo G. The expression of HSP60 and HSP10 in large bowel carcinomas with lymph node metastase. *BMC Cancer* 2005; 5:139.
- Cappello F, Di Stefano A, David S, Rappa F, Anzalone R, La Rocca G, D'Anna SE, Magno F, Donner CF, Balbi B, Zummo G. Hsp60 and Hsp10 downregulation predicts bronchial epithelial carcinogenesis in smokers with chronic obstructive pulmonary disease. *Cancer* 2006; 107:2417-24.
- Ullrich SJ, Robinson EA, Law LW, Willingham M, Appella E. A Mouse tumor-specific transplantation antigen is a heat shock-related protein. *Proc Natl Acad Sci* 1986; 83:3121-5.
- Menoret A, Bell G. Purification of multiple heat shock proteins from a single tumor sample. *J Immunol Methods* 2000; 237:119-30.
- Lee KP, Racz LE, Podack ER. Heat-shock protein-based cancer vaccines. *Hematol Oncol Clinics N Am* 2006; 20:637-59.
- Wang HH, Mao CY, Teng LS, Cao J. Recent advances in heat shock protein-based cancer vaccines. *Hepatobiliary Pancreat Dis Int* 2006; 5:22-7.
- Janetzki S, Palla D, Rosenhauer V, Lochs H, Lewis JJ, Srivastava PK. Immunization of cancer patients with autologous cancer-derived heat shock protein gp96 preparations: A pilot study. *Int J Cancer* 2000; 88:232-8.

