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CCR Translations

Clinical Cancer Research

Low-Level Inhibition of Hsp90 Forces Cells to Tip Their (Antigenic) Hand

Pramod K. Srivastava[1] and Margaret K. Callahan[2]

Low-level inhibition of hsp90 enhances the antigenicity of cells whereas high-level inhibition diminishes antigenicity. The mechanism(s) by which hsp90 determines antigenicity are only partially clear. Regardless, these observations have profound implications in protein trafficking and antigen presentation and suggest a novel way to enhance the potency of existing anticancer agents.

See related article by Jaeger et al., p. 6392

In this issue of *Clinical Cancer Research*, Jaeger and colleagues (1) report observations that turn many traditional ideas about hsp90 on their head, but which expand upon the original observations of Susan Lindquist (2, 3) in a remarkable new way, and with enormous implications for cancer therapy.

Many years ago, Lindquist made the surprising suggestion that hsp90, a hyper-abundant protein of the cytosol, helped to "potentiate" as well as "buffer" the effects of mutations (2). In a series of elegant articles, she and her colleagues posited that the effects of mutations could be made immediately evident, or masked, by the folding of the mutated proteins by hsp90, and that perturbations in the levels of hsp90, could then mask or reveal the consequences of mutations. In her own words, "Hsp90 can act in diverse ways to couple environmental contingency to the emergence and fixation of new traits" (2). Considering that mutations (and their expression) play a central role in evolution, this deeply insightful idea put hsp90 as a unique chaperone of the evolutionary process itself.

The hyper-abundance of hsp90 (i.e., more abundant than required for normal physiologic purposes) was an essential component of Lindquist's idea. Hsp90 is a component of multiple multi-protein complexes and is "thereby a "hub of hubs" in regulatory circuits" (3). This aspect of hsp90 also drew the attention of those who seek to target metabolic pathways of cells to treat cancers. Because the overwhelming majority of pathways are common to normal and cancer cells, it is generally difficult to treat cancers by such targeting without significant morbidities and mortality. The experimental use of multiple hsp90 inhibitors to treat cancers has thus far failed to make a significant clinical impact, although clinical studies are still in progress.

Hsp90 inhibitors of all stripes have been used in clinical trials and in studies with cells *in vitro* in high doses so as to elicit the maximum inhibition of hsp90 with acceptable toxicity. In fact, such inhibition of hsp90 has also been shown in previous studies, to elicit the heat shock response. Inhibition of hsp90 with such acute and high doses has also been shown in previous studies to interfere with antigen presentation. Here, Jaeger and colleagues (1) confirm and expand upon those immune-suppressive consequences of potent inhibition of hsp90, and more importantly, show that a less potent inhibition of hsp90 has the opposite, that is, immune-stimulatory effect.

Keeping in mind that hsp90 is hyper-abundant within the cytosol, and inspired by the ideas of Lindquist, Jaeger and colleagues asked what happens if the hsp90 levels within cells were reduced rather than completely extinguished. To do this, they developed a method to maintain continuous low plasma concentrations of an orally bioavailable hsp90 inhibitor (hsp90i). They examined the effect of low dose administration of hsp90i on tumor growth in a mouse model and observed that tumor growth was inhibited in mice subjected to sustained low dose inhibition of hsp90. Remarkably, this effect was lost in immunocompromised mice. In contrast to the killing of cancer cells by ablation of hsp90 sought by the acute and high dose inhibition of hsp90, the antitumor effect of low dose inhibition of hsp90 was clearly immunological! Consistent with this observation, tumor cells from hsp90i-treated mice showed increased expression of MHC I (1) as opposed to the decreased expression of MHC I on tumor cells treated with acute and much higher inhibition of hsp90 in previous studies (4). Expression of MHC I on tumor cells was also required for the antitumor effect of hsp90i. As another demonstration of the uniqueness of the pathway of low dose inhibition of hsp90, the increase in MHC I expression in tumor cells from hsp90i-treated mice was independent of IFNγ signaling.

Jaeger and colleagues probed the repertoire of MHC I–presented peptides in regular tumor cells with the corresponding repertoire in tumor cells from hsp90i-treated mice and made the intriguing observation that hsp90i-exposed cells present a very large number of unique peptides. Consistent with this result, they demonstrate changes in the proteasome composition of hsp90i-exposed tumor cells. How does inhibition of hsp90 to lower levels increase the repertoire of MHC I–presented peptides? Jaeger and colleagues do not probe this question experimentally but suggest very reasonably that "low dose hsp90i destabilizes subsets of client proteins, thereby increasing immunoproteasome substrate availability and increasing the abundance of peptides for MHC I loading" (Fig. 1A). We suggest an alternative model (Fig. 1B) but also note that the two models are not mutually exclusive. Hsp90

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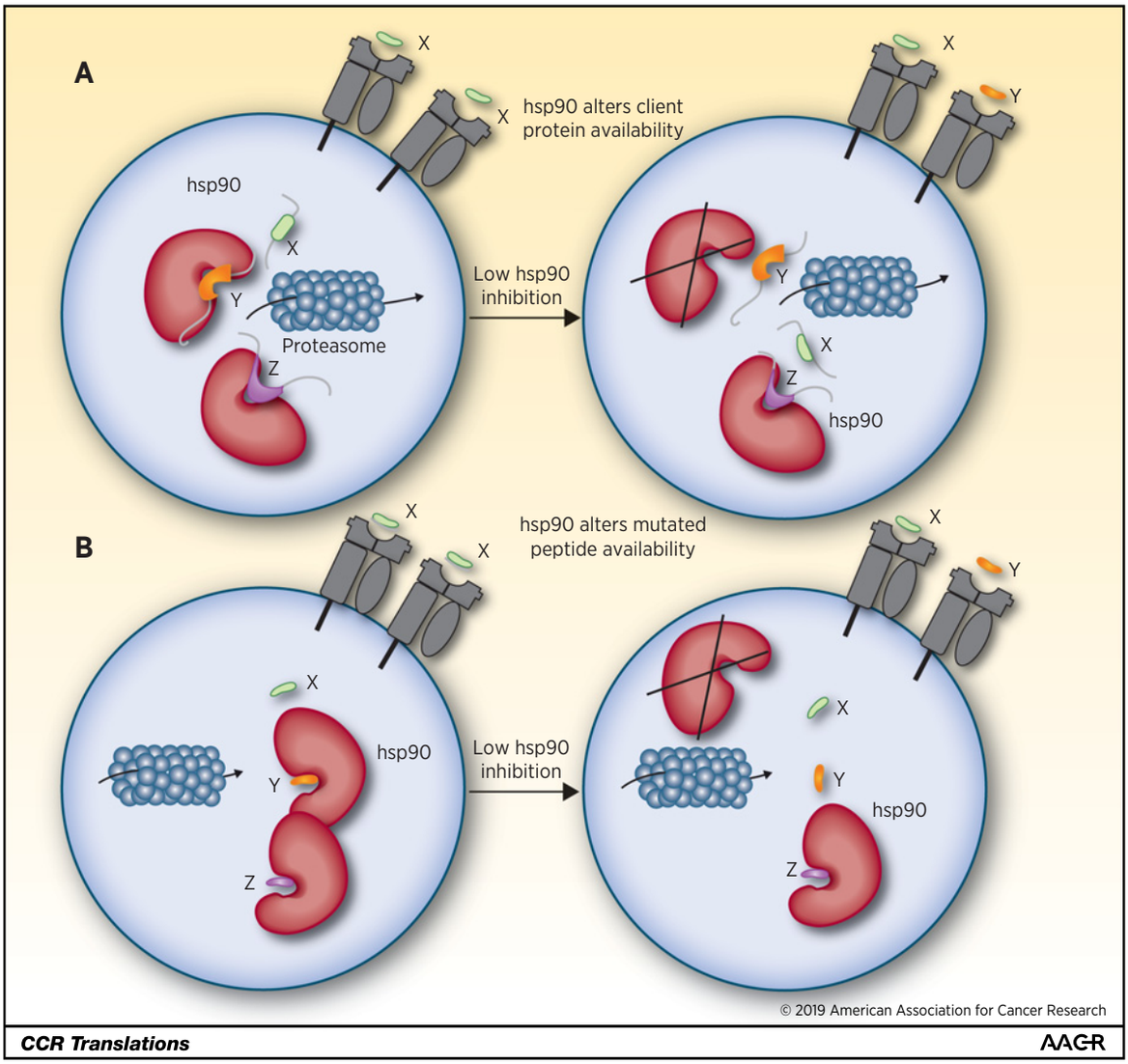

Figure 1.

Two models of how low-level inhibition of hsp90 reveals new antigens in cells. **A,** Low dose hsp90i destabilizes subsets of client proteins, thereby increasing proteasome substrate availability and increasing the abundance of peptides for MHC class 1 loading (1). X, Y, and Z represent epitopes within proteins. X is not a client protein of hsp90, whereas Y and Z are. Under normal conditions, only X is presented by MHC I and Y and Z are sequestered by being hsp90 bound. Upon low-dose inhibition of hsp90, Y is destabilized and subsequently available for proteasome processing and MHC class 1 presentation. Under these conditions, X and Y are presented on MHC class 1. Z exemplifies a client protein that is not destabilized under low dose inhibition of hsp90 and is not presented on MHC class 1. **B,** Hsp90–peptide association diverts postproteasomal peptides away from MHC class 1 antigen presentation, and inhibition of hsp90 relieves this inhibition, promoting a more diverse antigen presentation. Of the peptides X, Y, and Z, Y and Z bind hsp90 in the cytosol (4, 5) and are inhibited from presentation; only X is presented. Upon low-dose inhibition of hsp90, Y is relieved from association with hsp90 and is now presented, along with X. Z exemplifies a peptide that remains associated with hsp90 even under low-dose inhibition of hsp90 and is not presented.

has been shown to be associated with peptides including antigenic peptides (4). Indeed, such association of hsp90 with peptides has led to the suggestion that chaperoning of hsp90 with peptides is essential for antigen presentation (5). The results of Jaeger and colleagues suggest to us the opposite possibility: that hsp90–peptide association actually diverts peptides away from antigen presentation, and inhibition of hsp90 relieves this inhibition, promoting a higher and more diverse level of antigen presentation. All the previous data on association of hsp90 with antigenic peptides are consistent with this interesting and opposing hypothesis. In this opposing hypothesis, hsp90 can be seen to be buffering rather than facilitating antigen presentation. Regardless, the two hypotheses are not mutually exclusive and remain to be tested.

Jaeger and colleagues further demonstrate that a combination of treatment with hsp90i, leading to a higher and more diverse antigen presentation, with a nonspecific immune adjuvant mediates a stronger antitumor effect than either agent alone. This is consistent with their other results. Most interestingly for clinical translation, they suggest that currently used orally bioavailable

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hsp90is can be tested anew with the intention of moderately suppressing intracellular levels, and thus avoiding immune suppression and activation of the heat shock response, while at the same time promoting enhanced antigenicity of tumor cells. These finding also suggest that hsp90 functions to sequester a selective subset of mutated proteins and/or peptides that can function as antigens—the features that define these neoantigens are worthy of further study. This approach has the potential of revitalizing a substantial field of experimental cancer therapy. Like any important discovery, this approach does raise new questions mechanistically, as well as in terms of its translation. Appropriately, Jaeger and colleagues allude to the possibility that their approach may lead to autoimmunity although they find no evidence of it in treated mice.

Like all seminal ideas, Lindquist's idea on the role of hsp90 in buffering mutations has found a possible application in one of the most pressing problems of medicine. This was certainly not her goal, and this irony is a good reminder of the premise that scientific curiosity is best promoted and nurtured for its own sake. Important ideas get translated anyway.

Disclosure of Potential Conflicts of Interest

P.K. Srivastava is obligated by the University of Connecticut to declare financial interests in Truvax Inc., which has no connection with this article. M.K. Callahan reports receiving commercial research grants from and has immediate family members who are employed by Bristol-Myers Squibb, and is a consultant/advisory board member for Merck, AstraZeneca, Moderna, and InCyte. No other potential conflicts of interest were disclosed.

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