Chaperone substrate provides missing link for cancer drug discovery

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Edited by Norma M. Alliewell

Both Hsp70 and Hsp90 chaperones are overexpressed in cancer, making them relevant targets for the development of cancer chemotherapeutics, but a lack of biomolecular readouts for Hsp70 inhibition has limited the pursuit of specific inhibitors for this enzyme. A new study from Cesa et al. identifies two inhibitors of apoptosis proteins (IAPs) as specific client substrates of Hsp70. These results establish biomarkers that can be utilized to monitor Hsp70 inhibition and provide a framework for future efforts to deconvolute chaperone networks.

Heat shock proteins (Hsps)\(^2\) are molecular chaperones responsible for the conformational maturation of nascent polypeptides and the renaturation of denatured, misfolded, or aggregated proteins into their biologically active conformation (1). Many of the substrates, or clients, that depend upon the Hsp family member Hsp90 are critical to cancer survival, proliferation, and metastasis, and Hsp90 is overexpressed in many types of cancer. As a result, Hsp90 was the first molecular chaperone to represent a therapeutic target for the development of anti-cancer agents. It has now been shown that Hsp90 inhibition leads to the simultaneous degradation of \(>30\) oncogenic proteins (2), and Hsp90 inhibitors have been developed that can inhibit Hsp90 in transformed cells with high differential selectivity (3). In fact, seventeen compounds have undergone clinical evaluation for the treatment of cancer, but no Hsp90 inhibitor has been approved by the Food and Drug Administration due to toxicity and dosing difficulties observed during these trials (4). Although a few Hsp90 inhibitors remain under clinical evaluation, researchers are eager to explore other chaperones as putative drug targets. However, these studies have been stymied by a lack of knowledge about specific chaperone-client pairs, meaning that researchers could not be certain that the biological effects of a new inhibitor were directly caused by its inhibition of the target of interest. A new study by Cesa et al. (5) helps to solve this problem by identifying specific clients of Hsp90 that can serve as biomarkers in cellulo and in vivo, in the process raising new questions about the basis for Hsp70-Hsp90 interactions (5).

Hsp70, the 70-kDa member of the heat shock protein family, is also overexpressed in cancer. Moreover, it has been shown to be responsible for increased proliferation of cancer cells and the emergence of drug resistance (6, 7). Hsp70 works with other chaperones, such as J proteins, to bind nascent polypeptides that are either folded or transferred to Hsp90 for maturation into their biologically active conformation. This crosstalk has made it extremely difficult to identify Hsp70-specific clients, as inhibition of either Hsp70 or Hsp90 can have direct or indirect effects on the entire protein folding process. Recently reported Hsp70 inhibitors provide an opportunity to search for Hsp70-dependent clients (8, 9), which could then be used as biomarkers to improve our understanding of Hsp70’s role in cancer and aid in the development of more efficacious Hsp70 inhibitors.

The search for specific substrates by Cesa et al. (5) began with a focused list of candidate substrates based on prior studies. The researchers then compared the effects of the Hsp70 inhibitors JG-98 and PES to those of the Hsp90 inhibitors AUY-922 and 17-DMAG on MDA-MB-231 breast cancer cells to identify Hsp70-dependent clients. Western blot analysis revealed that the Hsp70 inhibitors induced the degradation of X-linked inhibitor of apoptosis protein (XIAP) and c-IAP by \(\sim 75\%\) within 6 h, whereas the Hsp90-dependent client, Raf-1, was only reduced by \(\sim 20\%\). The Hsp90 inhibitors, however, caused a strong (\(>95\%\)) reduction of Raf-1 within 12 h but no degradation of XIAP and c-IAP within 6 h, and only limited decreases after 24 h (Fig. 1) (5). Overexpression of IAPs in cancer is a prosurvival response that decreases programmed cell death, and its inhibition is highly sought after. These results demonstrate for the first time that XIAP and c-IAP exhibit selective dependence upon the Hsp70 chaperone system and provide an alternative method for IAP inhibition.

To further understand the relationship between IAPs and Hsp70, the authors performed co-immunoprecipitation experiments, confirming that XIAP binds Hsp70. Analysis of XIAP’s sequence suggested up to seven different Hsp70 sites might lie within the two “BIR” domains, or regions involved in blocking apoptotic signaling. Binding assays showed that a construct of XIAP containing those domains (XIAP(120–356)) bound Hsp70 quite tightly and that this interaction was significantly weakened upon inhibitor treatment. Interestingly, Hsp90 was able to competitively bind XIAP in the presence of Hsp70, despite the limited effect of Hsp90 inhibitors on XIAP levels observed in the earlier cellular experiments. Additional studies are needed to better understand the roles played by Hsp70 and Hsp90 in the activation of XIAP.
Next, the authors wanted to determine where XIAP binds Hsp70. ELISA and NMR experiments revealed XIAP does not bind Hsp70’s canonical substrate-binding domain, the region wherein the majority of proteins and polypeptides bind. Further experiments with a known client peptide (NRLLLTG) binding to Hsp70 produced ~15 new cross-peaks in the NMR spectrum that correspond with binding to the canonical region of the SBD, but the XIAP-bound Hsp70 spectrum did not produce such characteristic peaks, confirming an unexpected binding site. Additional studies showed that both the nucleotide-binding domain and substrate-binding domain competitively bind XIAP in the presence of Hsp70, albeit the nucleotide-binding domain binds with significantly lower affinity. These data suggest the XIAP-Hsp70 interaction will serve as an interesting case study to learn more about the possible mechanisms by which chaperones and their substrates interact.

In order to establish XIAP and c-IAP as suitable biomarkers for Hsp70 inhibition, the authors tested the inhibitors in three other cell lines, including cervical cancer (HeLa), breast cancer (MCF7), and normal human lung fibroblast (IMR90) cells. HeLa and MCF7 cells treated with the Hsp70 inhibitor JG-98, but not those treated with the Hsp90 inhibitor 17-DMAG, showed degradation of the IAPs at 6 h. In contrast, treatment of IMR90 cells with JG-98 did not reduce XIAP or c-IAP levels. Thus, Hsp70 inhibitors selectively target IAPs utilized in cancer and not normal cells. These results provide further evidence that Hsp70 is a promising target for the development of chemotherapeutics because such inhibitors could exhibit high differential selectivity toward cancer cells. Finally, when MCF7 tumor xenographs in mice were treated with JG-98, the isolated tumors contained significantly lower levels of XIAP and/or c-IAP than the control group, which demonstrates these IAPs are useful biomarkers in vivo as well.

The work from Cesa et al. (5) provides compelling evidence that XIAP and c-IAP are Hsp70-dependent clients and have demonstrated that at least XIAP can serve as a biomarker to monitor Hsp70 inhibition both in cellulo and in vivo. The authors provided some insight as to how XIAP and Hsp70 interact, but mechanistic questions remain. The most obvious question resulting from this work is what role does Hsp90 play in the XIAP protein-folding process? Further elucidation of the dynamic interplay between Hsp70 and Hsp90 will likely lead to the discovery of additional Hsp70-dependent clients, whereas identification of a biomarker for Hsp70 inhibition provides drug discovery researchers a powerful tool to initiate efforts toward the development of a new generation of chaperone inhibitors.

Acknowledgment—We thank Caitlin Kent for generating the figure.

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