HSP70 and FLT3-ITD: Targeting chaperone system to overcome drug resistance

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Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by the abnormal differentiation and rapid proliferation of hematopoietic cells. The causes of this type of blood cancer are multiple, with approximately 30% of AML patients characterized by expression of the internal tandem duplication (ITD) of the Fms-like tyrosine kinase 3 (*FLT3*) gene.[1] Despite the availability of effective FLT3 inhibitors, alone or combined with conventional chemotherapy, for the treatment of FLT3-ITD AML, there is a risk of leukemia relapse due to drug resistance. Reasons for drug resistance include the evolution of the secondary mutations of FLT3 in response to drug treatment and compensatory activation of survival signaling pathways.[2] Therefore, the discovery of new drug targets and novel therapeutic strategies for FLT3-ITD-positive AML is imperative for overcoming resistance to FLT3 inhibitors.

Due to drug resistance mechanisms, such as secondary mutations that render FLT3 inhibitors less therapeutically effective, selective degradation of FLT3-ITD may potentially provide a useful method for therapeutic intervention. In the latest issue of *Signal Transduction and Targeted Therapy*, Hu et al reported that targeting of heat shock protein 70 (HSP70), a chaperone protein, disrupted FLT3-ITD protein folding and consequently inhibited the proliferation of FLT3-ITD-positive and drug-resistant AML cells through the induction of proteasome-mediated degradation of FLT3-ITD.[3] Proteasome-mediated degradation of oncogenic proteins has emerged as an effective strategy in cancer therapy, including proteolysis-targeting chimera-mediated protein degradation through ubiquitin modification,[4,5] inhibition of the reverse biological process with a deubiquitinase inhibitor,[6,7] and targeting of the chaperone protein system that causes protein misfolding and clearance by proteasomes (Fig. 1).

The chaperone protein system includes foldases and holdases. Foldases support the folding of proteins in an ATP-dependent manner, while holdases bind folding intermediates to prevent their aggregation. Both HSP70 and HSP90 are foldases that cooperate to bind and facilitate the folding of their client proteins, thus maintaining cellular proteostasis against harmful environmental stress. Tumor cells are more dependent on HSPs than normal cells for proliferation because the oncoproteins in transformed cells require HSPs to stabilize or correct their structures.[8] Inhibition of HSPs can potentially disrupt multiple cellular signaling pathways essential to cancer cell survival. Therefore, targeting HSPs could be an attractive therapeutic approach for heterogeneous malignancies such as AML. Through high-throughput screening and proteomic analysis of compounds that inhibit the proliferation of FLT3-ITD-positive cells, Hu et al discovered that QL47 (Fig. 1), a previously reported irreversible BTK inhibitor, covalently targets inducible HSP70 protein without occupying its ATP-binding site.[3] This leads to a significant reduction of FLT3 protein levels and anti-tumor efficacy in vivo. Importantly, QL47 induced a robust decrease in reported mutations of FLT3 conferring drug resistance, suggesting that targeting of HSP70 may potentially be a promising therapeutic approach for the treatment of drug-resistant FLT3-ITD-positive AML.[3]

FLT3-ITD is a known client protein of HSP90, and thus degradation of FLT3-ITD through targeting of HSP90 represents a potentially effective approach to inhibiting FLT3-ITD protein function and thus blocking cell proliferation. HSP90 inhibitors, 17-AAG, which mimics the ATP binding site of HSP90, reached the clinical stage of development for AML; however, its unacceptable toxicity limited further development, and the unpromising clinical results presumably due to the simultaneous increase in HSP70 through the activation of HSF1.[9–12] Combination of 17-DMAG, a derivative of 17-AAG, with the HSP70 inhibitor, VER-155008, has been investigated and synergy between the two agents against primary AML cells in vitro was shown, suggesting combination therapy based on the chaperone protein system as a therapeutic strategy for AML.[13]

HSP70 has been reported highly expressed in many cancers and is associated with poor survival of patients diagnosed with chronic AML, AML, or malignant melanoma.[13] The chaperone system provides a physical platform for the binding of client proteins, other chaperones, and co-chaperones, such as HSP90, and facilitates oncogenic protein folding, maturation, disaggregation, and proteolytic pathways important for cancer survival.[14,15] Reported client proteins for HSP70 include inhibitors of apoptosis (IAP) proteins, including X-linked IAP protein (XIAP), the BCR-ABL fusion protein, the co-chaperones C-terminus of HSP70-interacting protein (CHIP) and Bcl-2-

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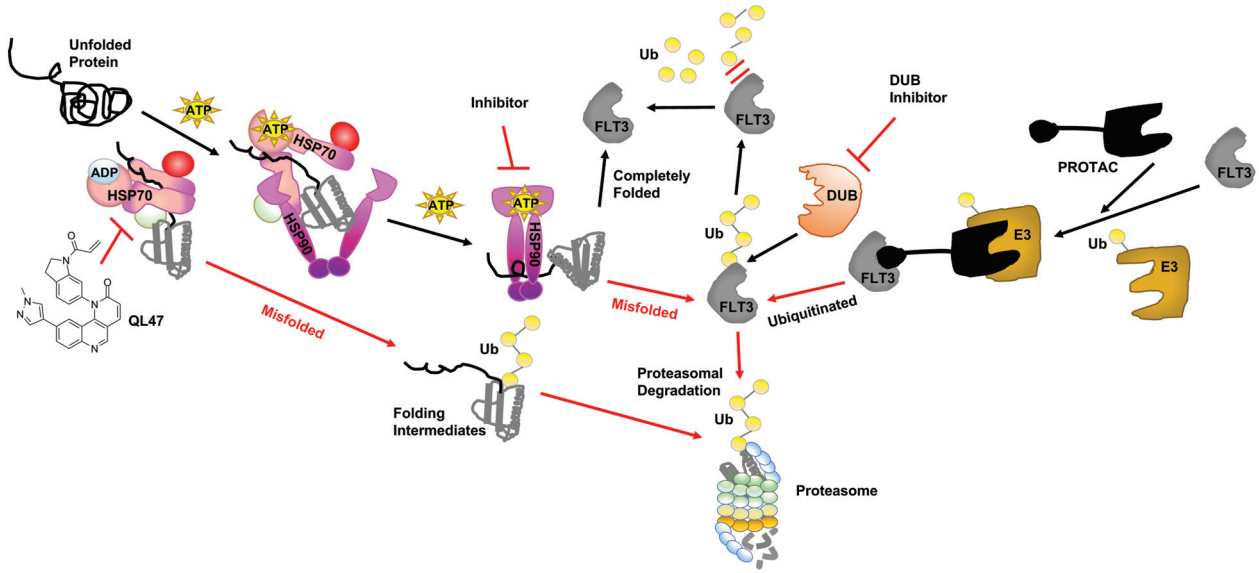

Figure 1. Proteasome-mediated degradation of oncogenic proteins. Three approaches to targeted oncogenic protein degradation through activation of proteasomal-mediated degradation are depicted: Targeting of the chaperone protein system, DUB inhibitor treatment, and the use of a PROTAC. DUB = deubiquitinase, PROTAC = proteolysis-targeting chimera.

associated athanogene 1 (BAG-1), and some newly identified proteins.[8,16,17] Interaction of HSP70 with different immunological regulators, such as the transcriptional factor of immunosuppressive regulatory T cell, FoxP3, reveals its potential immunotherapeutic activity.[18,19] Based on these observations, HSP70 is anticipated to be a potentially promising therapeutic target associated with the reversal of drug resistance.

However, due to the ubiquitous expression patterns of HSP70 and the complexity of HSP70 function, there are numerous challenges associated with designing efficacious and safe HSP70-based therapeutics. Small molecule inhibitors of HSP70 continue to represent the most common and promising therapeutic approaches that are based on targeting HSP70, and progress has been made in terms of developing HSP70 inhibitors that are more efficacious than earlier compounds. QL47 is an example of a class of HSP70 inhibitors that differs from previously reported canonical HSP70 inhibitors, such as VER-155008,[13] which compete for the ATP binding site of HSP70. Hu et al demonstrated that QL47 has a distinct mechanism of action that does not affect the ATPase activity of HSP70 however that translates into superior drug potency.[3] Another therapeutic approach involves designing antibodies against HSP70 or an HSP70-based recombinant cancer vaccine. A good example is the autologous heat-shock protein 70 peptide vaccine (AG-858), which is currently in clinical trials (NCT00058747). In addition to HSP70-targeting as a single agent strategy, combination therapy based on an HSP70 inhibitor or depletion of HSP70 with other co-chaperones, such as HSP90, offers a potentially more effective strategy for the treatment of many cancers expressing client oncoproteins, including FLT3-ITD-positive AML. Perturbation of the central survival signaling pathway and induction of apoptosis through FLT3 degradation could potentially overcome drug resistance and thus improve the treatment outcome for this subtype of AML.

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