Autophagy ›

Volume 7, 2011 - Issue 9

Free access

Autophagic Punctum

Development of p97 AAA ATPase inhibitors

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Pages 1091-1092 | Received 28 Apr 2011, Accepted 13 May 2011, Published online: 01 Sep 2011

Cite this article https://doi.org/10.4161/auto.7.9.16489

Abstract

Specific p97 inhibitors are valuable research tools to carry out mechanistic and cellular investigations of p97 biology. p97 is an abundant, ubiquitin-selective chaperone that has multiple functions and is essential for life. Therefore, genetic methods that require long incubations like siRNA or expression of dominant-negative p97 mutants are likely to generate complicated outcomes due to secondary consequences that arise upon slow depletion of p97 activity. We recently identified a small molecule p97 inhibitor, N^2,N^4-dibenzylquinazoline-2,4-diamine (DBeQ), and documented its effects on blocking autophagic degradation of LC3-II and proteasomal degradation of a p97-dependent ubiquitin-proteasome system (UPS) substrate. What distinguishes DBeQ from conventional proteasome inhibitors is that DBeQ affects both the UPS and autophagic protein degradation pathways and rapidly activates cell death. Whether DBeQ activates autophagic and/or apoptotic cell death will require further work to evaluate its detailed mechanism of action. An exciting goal for the future will be to generate p97 inhibitors that affect one or the other pathway. We propose that generation of ‘separation of

function' inhibitors will be a challenging adventure for chemical biologists but will yield extremely powerful tools to study p97 and enable evaluation of the therapeutic potential of targeting distinct p97 complexes.

This article refers to:

p97 AAA (ATPase associated with diverse cellular activities) ATPase is known as valosin-containing protein (VCP) and translational endoplasmic reticulum ATPase (TERA). p97 plays critical roles in a broad range of cellular activities, which is thought to derive from its ability to associate with different cofactors and interacting partners. Various cofactors link p97 to specific functions in many different cellular processes. In general, it is thought that p97 unfolds proteins or remodels/disassembles protein complexes, but the detailed mechanism of how p97 works and is linked to specific cellular processes remains unknown. Recent proteomic network analysis of the human autophagy systems reveals interaction of p97 with 17 autophagy-related proteins. This finding hints at multiple unidentified functions of p97 within the autophagy pathway.

p97 is most well-studied for its role in mediating degradation of ubiquitinated proteins via the endoplasmic reticulum-associated degradation (ERAD) pathway, in which misfolded proteins are extracted from the endoplasmic reticulum and ultimately degraded by the proteasome. More recently, p97 has been implicated in degradation of aggregated proteins through the aggresome-autophagy pathway. Given that a key mechanism of action of proteasome inhibitors in cancer therapy is thought to be their ability to block ERAD, and given that autophagy is induced as a backup mechanism in response to proteasome inhibition, we hypothesized that inhibition of p97 might be an effective approach to kill cancer cells. Blockade of both degradation pathways may cause rapid protein imbalance culminating in a lethal unfolded protein response. This may have particularly dire consequences for cancer cells that are aneuploid, and therefore prone to produce unbalanced levels of subunits of protein complexes.

A high-throughput screening (HTS) effort performed in collaboration with the NIH Molecular Libraries Probe Centers Network (MLPCN) yielded several inhibitors of p97 ATPase activity. Among them, N^2,N^4-dibenzylquinazoline-2,4-diamine was identified as a selective, reversible and ATP-competitive p97 inhibitor. Importantly, DBeQ exhibits 20-fold selectivity for stabilizing p97-dependent vs. independent UPS reporter substrates in

HeLa cells. Some examples of previously known p97 substrates in mammalian cells are grouped into 4 categories in Figure 1. DBeQ impairs degradation of substrates within the ERAD and autophagy pathways. A lot of questions remain to be addressed. Does DBeQ target all p97-dependent processes or only a subset of them? Evaluation of the effects of DBeQ on other p97 functions will provide more insight into the full spectrum of its activity.

We now focus our effort on determining how DBeQ activates caspase 3/7 and induces cell death so rapidly. An important question is, does induction of cell death by DBeQ occur through autophagic and/or apoptotic machineries? DBeQ completely inhibits autophagic degradation within 30 min. By contrast, caspase 3/7 activation is prominent after 4 h treatment. These kinetics are consistent with the possibility that inhibition of autophagy underlies the cell-killing activity of DBeQ. An alternative possibility for why DBeQ induces rapid caspase activation is that DBeQ may impinge on maintenance of inhibited caspase-IAP (inhibitor of apoptosis) complexes. It is possible that the caspase activation and cell death induced by DBeQ are ‘off-target’ effects mediated by unknown factors. However, p97 knockdown also induces caspases 3 and 7 and cell death.

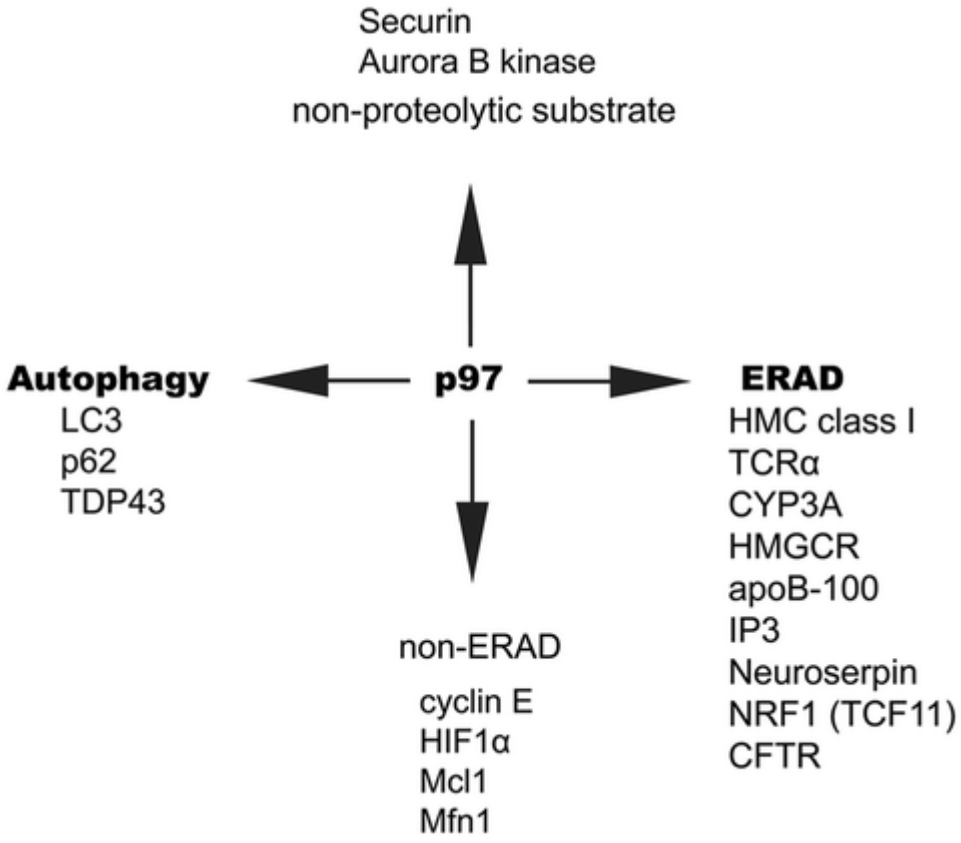
Currently, an extensive structure-activity relationships (SAR) study is underway to discover more potent and specific p97 inhibitors. Simultaneously monitoring the effect of novel p97 inhibitors on purified p97, cell-based p97-dependent and independent UPS reporters, autophagic markers, caspase activation and other unexplored substrates will enable us to classify their effect on different p97 pathways. We are optimistic that this effort will generate new and better inhibitors, and may even yield compounds that selectively inhibit specific subsets of p97 functions. New inhibitors will enable evaluation of the therapeutic potential of targeting p97 in various diseases, such as cancer, neurodegenerative disease and protein-folding disorders.

Abbreviations

LC3	=	microtubule-associated protein 1 light chain 3
ERAD	=	endoplasmic reticulum associated degradation

Figures and Tables

Figure 1 Examples of p97 substrates. Most known p97 substrates belong to the ERAD pathway. Emerging data suggests that p97 is involved in degradation of many other non-ERAD UPS substrates and autophagic substrates, as well as in disassembling and maintaining protein complexes (non-proteolytic function). DBeQ blocks the autophagy and ERAD functions of p97. More experiments are needed to determine whether DBeQ acts on other p97 pathways. Blockade of multiple pathways at the same time is likely to result in pleiotropic effects that resist molecular dissection. Therefore, development of 'separation of function' p97 inhibitors would provide more powerful tools to study individual p97 functions in cells.



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Acknowledgements

We thank H. Park, R. Oania and D. Shimoda for technical assistance. T.F.C. was supported by a 2008 Fellows Grant Program Award from the Multiple Myeloma Research Foundation, the Howard Hughes Medical Institute (HHMI) and the Weston Havens Foundation. R.J.D. is an HHMI Investigator, and this work was funded in part by HHMI and in part by a National Institutes of Health R03 grant (MH085687).

Punctum to: Chou TF, Brown SJ, Minond D, Nordin BE, Li K, Jones AC, et al. Reversible inhibitor of p97, DBeQ, impairs both ubiquitin-dependent and autophagic protein clearance pathways. Proc Natl Acad Sci USA 2011; 108:4834 - 4839; PMID: 21383145; http://dx.doi.org/10.1073/pnas.1015312108