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The Next Big Thing In Drug Development May Be The Ubiquitin Pathway

Velcade, Millennium's successful first-in-class cancer therapy, is held up as a poster drug for manipulating the ubiquitin system to attack disease. Yet it taps only the most rudimentary process of an elegant and little understood cellular mechanism that some are betting will be the next big source of innovation in drug development.

The ubiquitin proteasome pathway is not well-characterized, though it is the best known of a complex system of mechanisms whose disregulation is thought to be involved in diseases such as cancer, neurodegenerative disorders, immune and inflammatory responses, musculoskeletal disorders and viral and bacterial infections.

In fact, it is the stunning complexity of this cellular system, which regulates the lifetime and intracellular distribution of proteins and, thereby, a variety of signal transduction and other physiological mechanisms, that invites therapeutic intervention. There are so many moving parts and variations that mapping the possibilities has been likened to the Human Genome Project.

Ubiquitin is a polypeptide chain that is attached by enzymes called proteases to proteins targeted for degradation by the proteasome, a cellular complex often compared to a garbage disposal. Ubiquitins carry their load into the proteasome and are themselves recycled to repeat the process. The system is at once simple and elegant, and the tagging is reversible.

Ubiquitins can work alone or in more complex chains connected by their lysine residues (think glue). The combinations have yet to be fully mapped and defined among the seven possible lysines, but the nature of the ubiquitin linkage determines the fate of the target protein, and polyubiquitin chains are used for more than taking out the garbage. They are involved in DNA repair, cell signaling, stress response, membrane trafficking and a host of other functions that are only dimly understood. The most well-characterized linkage, however, is the one that builds chains using lysine-48 to carry proteins to the proteasome to be degraded.

Velcade (bortezomib) is a proteasome inhibitor; it prevents the degradation of poly-ubiquitylated proteins. The resulting cytotoxicity makes it a very useful, if somewhat indiscriminate, tool against certain blood cancers.

The ubiquitin enzymatic cascade is responsible for initiating and directing the linking process of ubiquitin to its target protein. An activating enzyme, E1, primes the ubiquitin and connects it to an E2, or conjugating enzyme, which then facilitates its attachment to the target using an E3 ligase, or E3. E3 ligases are slippery drug targets because a large majority have no active sites for small molecule intervention, but they are nonetheless inviting thanks to their position in the cascade and specificity for target identification.

There are two known E1s, around 40 known E2s, and around 600 E3 ligases, as well as around 100 de-ubiquitylation enzymes – specific proteases that can remove ubiquitin from a target and save it from degradation. Altogether, they provide a host of potentially drugable targets.

Comparison With Kinases Is Inevitable

The ubiquitin system frequently draws comparisons to kinases as the next big intracellular system to be mined for interventions. But the ubiquitin field is much more intricate, according to Jeffrey Marblestone, senior scientist, and Craig Leach, associate director, at Progenra, a Malvern, Pa., biotech that subtitles itself "The Ubiquitin Company."

Like the ubiquitin system, kinases control almost all areas of cell biology. There are over 500 kinases, also known as phosphotransferases, which transfer phosphate groups from the cell's energy transport nucleotide, adenosine triphosphate (ATP), to molecules, including proteins, through a reversible process called phosphorylation.

But the reversible phosphorylation of proteins is a much more general mechanism for regulating cell functions than manipulating the ubiquitin system. Essentially, kinases can add the phosphate to the target, and phosphatases can remove it, and with ubiquitin, E3 ligases can attach ubiquitin to a target and ubiquitin proteases can remove it, but that's where the functional similarities end.

With ubiquitins, one ubiquitin attached to a target gives one message whereas a chain can give another and then there are the various types of poly-ubiquitin chains possible. Additionally ubiquitin's "cousins" – the ubiquitin-like proteins – can also be attached to target proteins, using their own specific ligases and proteases. While ubiquitin is the most studied, these other ubiquitin-like molecules also hold therapeutic potential, Leach explained.

"Ubiquitin is a more complex system than phosphorylation. There are flavors of ubiquitylation whereas with phosphorylation there is only one flavor," he said.

One thing ubiquitins do have in common with kinases is a long lag time between their discovery and the recognition that the field is an important area for drug discovery, said Sir Philip Cohen, a pioneer in protein phosphorylation and now a champion of the ubiquitin system.

After its slow uptake, kinase research now accounts for around 30 percent of all research in pharma, and more like 50 percent to 70 percent of cancer drug discovery. There are approximately 380 compounds in development or marketed, and they are expected to bring in $50 billion in 2010.

Phosphorylation was discovered in 1955. It garnered its discoverers a Nobel Prize in 1992, and the first kinase drug, Novartis' tyrosine kinase inhibitor ***Gleevec*** (imatinib), was approved in 2001. For ubiquitylation, the time from discovery in 1978 to the Nobel in 2004 was shorter, around 26 years, and the first therapy was also approved much sooner. Velcade was approved in 2007.

Academy-Industry Collaboration In The U.K.

Things will move faster for the ubiquitin system, predicts Cohen, who is at the University of Dundee, Scotland, and has received government funding to start a protein ubiquitylation unit there modeled on the phosphorylation unit he's run since 1990. The Scottish Institute for Cell Signaling (SCILLS) Protein Ubiquitylation Unit was launched in October 2008.

For one thing, scientists have overcome their "inherent distrust" of drugs that target proteins inside the cell, Cohen said. Even though very little is going on in the industry in terms of direct ubiquitin programs, "I'm amazed at how many phone calls and e-mails I'm getting from biotech and pharma companies all over the world," Cohen said. "They don't know enough to go into it, so they're looking for advice."

Cohen's original kinase-focused unit is part of an academia/industry collaboration begun in 1998 to speed up development of protein kinase inhibitors with therapeutic potential. The alliance currently includes AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, Merck-Serono and Pfizer. Members share all unpublished results and the first rights to license the intellectual property filed by the unit. Work the unit performs for individual members remains private. The consortium has contributed $60 million in research funding over 11 years, "which has allowed us to do things in a way that wouldn't have been possible with the normal levels of government funding for basic research," Cohen said.

"We've helped to launch and accelerate literally hundreds of drug discovery programs in this area, and we've set up technologies like kinase profiling that have had an impact on lead optimization in the area," Cohen said. Buoyed by the success of the kinase effort, Cohen said he decided it was time to set up a unit devoted to ubiquitins.

The Scottish government's boost started the new unit with a five-year, $16 million to $17 million commitment, and Cohen is in the process of filling out five research teams. Three of the teams – protein production, assay development and cloning – are in place, and "I'm rapidly getting a saturation coverage of all the proteins of the ubiquitin system," he said.

Millennium Remains The Ubiquitin Frontrunner

With the proteasome validated as an oncology target by Velcade, Millennium (now the Takeda Oncology Company) continues working on inhibitors of enzymes within the ubiquitin system.

Velcade had $1.4 billion in 2009 sales. It is approved to treat multiple myeloma and in second line treatment of mantle cell lymphoma. But first-generation Velcade represents a ham-handed approach to proteasome regulation; it stops nearly all ubiquitylated proteins from being degraded, and that lack of specificity leads to side effects, such as neuropathy. The company's more-targeted second-generation protease inhibitor, MLN9708, entered a Phase I clinical trial in November in an oral formulation; an intravenous formula started Phase I in March 2009.

Millennium is taking a different tack on the ubiquitin system with its second clinical candidate, MLN4924. That molecule works upstream of the proteasome by inhibiting the Nedd8 activating enzyme (NAE), a ubiquitin-like protein that is part of the E1 family.

By inhibiting only Nedd8 conjugation, MLN4924 knocks out only certain ubiquitin E3 ligases, some of them responsible for cell cycle, oxidative stress response, DNA replication and repair. By silencing particular E3 ligases, the compound can inhibit cell proliferation and induce cell death. Preclinical studies showed that NAE is overexpressed in primary colon, lung and ovarian tumors, as well as in colon cancer metastasis to the liver, but has low expression in normal human tissues, giving it a lower potential for adverse effects. MLN4924 entered Phase I in 2008.

Millennium has an ongoing sponsored research agreement with Harvard Medical School in what it calls "protein homeostasis," to further elucidate the science in the ubiquitin space.

Interest Growing At Other Companies

On March 3, Boston-based biotech Stemgent announced it had formed a biotech to spin off products of Cohen's Scottish ubiquitin endeavor. Employees of the new firm, Ubiquigent, in Dundee, have been working with scientists at SCILLS for four months, Stemgent said. The new company is an "exciting and gratifying" turn of events, Cohen said.

Perhaps the best known ubiquitin candidate outside of Millennium's portfolio is carflizomib, the Phase IIb proteasome inhibitor Onyx Pharmaceuticals gained with its purchase of Proteolix in October. Onyx has set an aggressive timeline for development of the drug, which it says has a cleaner safety profile than Velcade ("The Pink Sheet" DAILY, Dec. 7, 2009).

Cephalon's investigative proteasome inhibitor CEP-18770 is in a Phase I/II study in patients with relapsed and refractory multiple myeloma that is expected to render data in late 2013, according to Inteleos, Elsevier's drug development database.

Nerus Pharmaceuticals has a proteasome inhibitor that comes from a marine-derived actinomycete bacteria. The molecule, NPI-0052, is in Phase I studies against both blood cancers and solid tumors.

Rigel Pharmaceuticals also has a ubiquitin ligase inhibition program, with oncology candidates licensed to Daiichi-Sankyo and Merck.

And French drug maker Hybrigenics has a program aimed at protease inhibition. The company has a platform in the molecular cell biology, enzymology and pharmacology of ubiquitin-specific proteases and a patent portfolio covering screening assays and original small-molecule inhibitors. It has two compounds in discovery: HBX-99200 is an ubiquitin-specific protease-8 inhibitor, and HBX-41108 inhibits ubiquitin-specific protease-7.

The tumor-suppressor protein p53 is regulated by USP7. Hybrigenics believes its small molecule drug can inhibit USP7, resulting in increased circulating p53 and inhibition of cancer cells.

Also working on an inhibitor of p53 destruction, Johnson & Johnson has JNJ-26854165, which acts against HDM2, an E3 ligase. The experimental molecule stops the HDM2-p53 complex from attaching to the proteasome, so p53 can't be degraded.

Ark Therapeutics is repurposing a proteasome inhibitor originally developed as a treatment for high blood pressure and currently marketed in Japan and European countries as ***Tanatril*** (imidapril). Renamed ***Vitor***, the compound now is in Phase III testing for treatment of muscle wasting in patients with non-small cell lung cancer.

The company is actively seeking a partner to heft the remaining development costs and share in commercialization. The compound increases the ability of mitochondria to produce energy and stops protein breakdown in muscle cells, according to Ark.

Privately held Progenra has proprietary target and drug discovery platforms as well as lead optimization capabilities. "What we've done is develop technologies to help us characterize a lot of these enzymes, and we are using it to search for our small molecule inhibitors," explained Marblestone.

For example, Progenra has used its reagent platform to identify P5091, a selective small molecule inhibitor of USP7 with in vivo efficacy in mouse models of cancer. In addition, the firm has earlier stage programs targeting other de-ubiquitylating enzymes and ubiquitin E3 ligases. Progenra is interested in talking to potential partners about development of P5091 and other small molecules identified using its internal discovery program.

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Ubiquitin System-Targeted Drugs In Development

The Race Is On To Best Millennium's First-In-Class ***Velcade*** (bortezomib)

Sponsor	Compound	Status
Ark Therapeutics	***Vitor*** (imidapril) (repurposed ***Tanatril***)	Phase III
Onyx Pharmaceuticals	Carflizomib	Phase IIb
Cephalon	CEP-18770	Phase I/II
Millennium	Velcade follow-on MLN9708	Oral Phase I trial started 11/2009; I.V. form started Phase I 3/2009
Millennium	MLN4924	Phase I (2008)
Nerus Pharmaceuticals	NPI-0052	Phase I
Hybrigenics	HBX-99200 and HBX-41108	Discovery
Progenra	P5091	Discovery
Johnson & Johnson	JNJ-26854165	Unknown
Rigel Pharmaceuticals	Oncology candidates licensed to Daiichi-Sankyo and Merck	Unknown