Aileron Staples Peptides

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Existing drugs can be roughly classified either as biologics or small molecules. Biologics include antibodies or insulin that does not penetrate into cells, which limits their target range, as <10% of human proteins are found on cell surfaces or are secreted. Although able to penetrate cells, small molecules have limitations of their own. "The entire biotech industry has been working on 10% of human targets," said Gregory Verdine, Ph.D., Erving professor of chemistry, Harvard University. "Small molecules only work on proteins that have a specific feature on their surfaces. That is another 10% of all human targets. So that means 80% are not approachable by the two established modes of drugs."

[Aileron Therapeutics] is going after what were previously considered undruggable targets

Wielding the Chemical Staple Gun

Verdine and Cambridge, MA, based Aileron Therapeutics (http://www.aileronrx.com) believe that they have found the key to accessing this so-far untapped universe of targets. Proteins, through their subdomains, play a role in almost every cellular process in the body; however, subdomain-derived peptides have been difficult to turn into drugs. Because they lack defined structure, these peptides are quickly degraded by proteases and are dispatched by the kidneys. Currently, there are more than 40 peptide drugs on the market, such as Amylin's Byetta, an α-helical peptide treatment for diabetes, but the drugs target receptors on the cells' surfaces.

Aileron Therapeutics stabilizes peptides by "stapling" them with hydrocarbon bonds into an α helix, a structure peptides can naturally assume in proteins. Thus constrained in a helical formation, the peptides are protected from the depredations of proteases. The stabilized α-helical peptides can penetrate cells by energy-dependent active transport and usually have a higher affinity to large protein surfaces.

The company believes its stapled α-helical peptides have wide therapeutic potential for cancer, autoimmune, inflammatory, infectious, and metabolic diseases. Aileron was started in 2005 by CEO Joseph Yanchik, III, using IP licensed from Verdine at Harvard and cofounders Loren Walensky, M.D., Ph.D., assistant professor of pediatrics, and the late Stanley J. Korsmeyer, M.D., Sidney Farber professor of pathology and professor of medicine, at the Dana-Farber Cancer Institute and Harvard Medical School.

Aileron Therapeutics is funded by the investment arms of four pharmaceutical companies. In June 2009, it announced a $40 million D funding round from Apple Tree Partners, SROne, Ltd. (GlaxoSmithKline), Excel Medical Fund, Novartis Venture Fund, Lilly Ventures, and Roche Venture Fund.

Breaking Down Barriers for α-Helical Peptides

Chemically constraining peptides into a helix has challenged researchers for decades. Verdine's lab applied the catalyst developed by Nobel laureate Robert Grubbs, Ph.D. at CalTech. "We knew that α helices were frequently involved in mediating protein-protein interactions," Verdine said. "We also knew by maximizing helix stability we would solve the problem of proteolytic degradation. No protease can recognize an α helix." But, according to Verdine, stabilized α-helix peptides previously developed by other research labs did not penetrate cells.

To stabilize peptides, Verdine and his postdoc Christian Schafmeister, Ph.D. (now associate professor, Department of Chemistry, Temple University) used an olefin metathesis reaction. They drew upon the work of Isabella Karle, Ph.D, the former chief scientist at the Office of Naval Research, X-Ray Diffraction Section, who showed that adding α methyl groups to the peptides could locally stabilize the rings of the helix, and of Peter Schultz, Ph.D, professor of chemistry, Scripps Research Institute, who showed that macrocycle rings connecting adjacent turns of the helix created a global stabilizing effect. Staple placement also mattered.

Proteins can't penetrate a cell wall because they have too many polar atoms and are generally hydrophilic, thus, they are incompatible with the hydrophobic interior of the membrane. This led to the idea that the macrocycle ring should be all hydrocarbon. "We didn't want any atoms to impede cell penetration," said Verdine. Loren Walensky, then a postdoc in Verdine's lab, discovered that the stabilized cell helices didn't penetrate the cell via passive diffusion, but by active endosomal uptake. "That was a serendipitous but very, very important discovery," said Verdine. "What is so great about it is that it has broad applicability to a wide variety of peptides."

According to Verdine, bioavailability was another hurdle. For instance, when antisense oligonucleotides are taken up into the cell by endosomal trafficking, they can't get out of the endosome; however, stapled helixes are excellent escape artists. For example, the cell's nuclear envelop blocks passage of anything over 25 kDa, but allows smaller molecules through. Stapled α-helical peptides are small enough to access targets in the nucleus and in the cytoplasm. The synthetic peptides have a similar binding specificity to the protein from which they were derived.

Playing Cell Guardians and Executioners Off Each Other

Loren Walensky's laboratory is developing new strategies to study and treat

childhood malignancies, focusing in particular on recurring cancers that are unresponsive to existing treatments. Walensky researched α-helical stapled peptides to investigate and modulate cell death pathways as a postdoctoral fellow in the laboratories of Verdine and Stanley Korsmeyer, who was a pioneer in apoptosis research.

The Bcl-2 family of proteins regulates apoptosis, interacting in Byzantine fashion to dictate the life and death of cells. In a cancerous cell, the balance among Bcl-2 proteins goes haywire, blocking signals that initiate cell suicide. "A key interaction element for this family is the conserved BH3 death helix," said Walensky. "There are two basic classes of regulators: survival proteins and death proteins. Our goal is to use this natural helical motif to block survival proteins, shutting down their protective force field, and directly activate the death proteins."

Cancer cells may be more susceptible to pharmacologic death triggers, according to Walensky, because their survival proteins, while successful at maintaining cancer cell immortality, are barraged with internal and external stress signals. His current working hypothesis is that targeting the overtaxed survival pathways of cancer cells would be enough to tip them over the edge, while sparing normal cells.

In a 2004 *Science* paper, Walensky, Verdine, and Korsmeyer used stapled BH3 α-helical peptides to induce apoptosis of cancer cells, reducing the proliferation of transplanted cancer in mice (Walensky et al., 2004). In a 2008 *Nature* paper, Walensky and colleagues discovered how the BH3 helix of the proapoptotic Bcl-2 protein BIM directly binds and activates the BAX protein to trigger apoptosis (Gavathiotis et al., 2008). "The Bcl-2 family interaction network is as fascinating as it is complex," Walensky said. "Deploying stapled peptides as molecular probes and prototype therapeutics is leading us to new biology and hopefully new drugs to treat disease."

Collaborating with Walensky, Nika Danial, Ph.D., assistant professor of pathology at Harvard, discovered that the proapoptotic Bcl-2 protein BAD toggles between regulating apoptosis and metabolism by turning its death helix on and off. "Findings like this teach us to stay open-minded, since Bcl-2 proteins may have unanticipated functions outside the apoptotic pathway," Walensky said.

At the 2008 American Association for Clinical Cancer Research (AACR) annual meeting, Aileron debuted a study showing that stapled α-helical peptides based on the BH3 domains of proapoptotic BID and BIM proteins dose-responsively suppressed cell growth of various cancers in vitro and in mice. Owing to their structural stability, the peptides survived in the body for several hours.

A Tale of Two Molecules

Abbott Laboratories' ABT-263 is a rival compound to regulate apoptosis for cancer treatment. ABT-263 is currently in phase I/II clinical trials run by Genentech and Abbott for various cancer indications.

In 1996, Stephen Fesik, Ph.D, and his team at Abbott Laboratories determined the 3D structures of proteins involved in apoptosis, including the structure of a Bcl-2 family protein, Bcl-X_L. According to Fesik, while apoptosis is triggered in damaged cells, in some cancer cells the apoptosis signal becomes deregulated and the cell doesn't die. The team published papers in 1996 (Muchmore et al., 1996) and 1997 (Sattler et al., 1997) describing how the antiapoptotic protein Bcl-x_L interacted with a peptide derived from BAK, a proapoptotic member of the same family. "We were able to define what were the hotspots and important interactions that stabilized complex formation," Fesik said. "We thought we could come up with a small molecule that could essentially mimic the BAK peptide."

Fesik's team used NMR to identify fragments of the ultimate molecule they wanted to make and painstakingly constructed it. The resulting molecule, ABT 737, which was published in *Nature* in 2005, required IV administration. The team used medicinal chemistry to develop an orally active analog called ABT 263 (Oltersdorf et al., 2005).

"ABT-263 potently inhibits Bcl-x_L, Bcl-w, and Bcl-2," said Fesik. "In addition, ABT 263 regresses tumors in animal models and is showing promising anticancer activity in early stage clinical trials. However, ABT 263 does not inhibit Mcl-1. In certain cancers, Mcl-1 drives the antiapoptotic response, so for patients with high levels of Mcl-1, ABT 263 would have to be delivered in combination with other drugs."

According to Fesik, while developing a drug using stapled α helices is potentially easier, as the α-helical form already occurs in nature and just needs to be modified, Aileron now has to further prove the methodology. "They have to demonstrate the promise of this technology in clinical trials," said Fesik.

Fesik's accomplishment was a "tour de force in making a small molecule," said Tomi Sawyer, Ph.D., chief scientific officer at Aileron Therapeutics. "It was elegant and leveraged a fragment-based approach to small molecule lead generation and optimization." According to Sawyer, ABT-253 is designed for a specific target, while α-helical stapled peptides can be redesigned to achieve desired specificities for various targets. Aileron is developing a series of stapled peptides particularly effective against Mcl-1.

According to Sawyer, stapled peptides have target selectivity based on natural helical protein-protein interactions that is not readily achieved by small molecules. Cell penetration mechanisms for stapled peptides involve active transport processes, compared to small molecules which mainly penetrate into cells by passive diffusion. "Our first challenge was to get past the in vitro testing to understand if there are any limitations of stapled peptides' PK properties," said Sawyer. So far, Aileron has optimized the half-lives of key stapled peptides to 24 hr and is evaluating candidates in vitro and in vivo.

"It is an innovative modality with supporting data, albeit early stage," said Michael Diem, M.D., partner at SROne. According to Diem, while the Abbott molecule is the most advanced so far in the Bcl-2 pathway, what makes Aileron Therapeutics interesting to the pharmas is its breadth of platform. "It is going after what were previously considered undruggable targets," Diem said.

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