Innovations

Going in for the kill
Idun Pharmaceuticals, Inc.

Chemistry & Biology March 1999, 6:R81–R82

 ISSN 1074-5521

The body, it seems, is over-cautious. Faced with the insult of a stroke, heart attack or neurodegenerative disease, it will sacrifice cells that have minimal damage and could easily be rehabilitated. Idun Pharmaceuticals, Inc. (La Jolla, California) believes that it can intervene in this process by turning off the programmed cell death, or apoptosis, that kills the cells. The company also aims to trigger apoptosis in cancer cells. Both projects are focused on small-molecule drugs, but they will be helped by Idun's licensed technology for getting proteins into cells — a technology that will, at least according to its inventor, revolutionize the pharmaceutical industry.

Move fast and recruit aggressively

In November 1993, Robert Horvitz of the Massachusetts Institute of Technology (Cambridge, Massachusetts) announced the cloning of CED3, an apoptosis-promoting gene from the worm *Caenorhabditis elegans*. CED3 was most similar to human interleukin-1 converting enzyme (ICE), which cleaves the pro-inflammatory messenger interleukin-1 to yield its active form.

Horvitz and his collaborators were not put off by this odd similarity, and in 1993 they formed the cell-death company Idun. An early experiment showed that an ICE inhibitor could, indeed, block neuronal apoptosis.

John Reed of the Burnham Institute (La Jolla, California) was looking at the flip side of apoptosis — the anti-death gene Bcl-2 — but he came to the same conclusion as Horvitz: this field needed a biotech company. Reed's company was named Apoptech Inc., but it had a brief life. "The two companies were talking to the same people for the same licenses, so they merged in 1994 to form Idun," says Steven Mento, now president and CEO of Idun.

The combined company quickly lined up a stellar scientific advisory board, which includes Horvitz, Reed, Stanley Korsmeyer (Harvard Medical School, Boston, Massachusetts), Craig Thompson (University of Chicago, Illinois), Martin Raff (University College London) and Carlo Croce (Jefferson Medical College, Philadelphia, Pennsylvania). And with collaborative research agreements and aggressive licensing, Idun now has issued or pending patents for Bcl-2 and related proteins, and for six of the cell-death proteases, or caspases (caspases 3, 6, 7, 8, 9 and 10).

"They have been at the forefront of some of the advances in this field," says Scott Kaufmann (Mayo Clinic, Rochester, Minnesota), who is not affiliated with Idun. "They have selective inhibitors that nobody else has, and they have been more than keeping up with the enzymology as investigators clone new caspases."

The case for caspase therapy

If cells are broken open by a crush, burn or chemical injury, or if they are completely deprived of oxygen, they die by necrosis. Thus the cells at the center of a region of stroke or heart attack — cells that have had their oxygen supply completely cut off — will mainly die of necrosis. But cells that are close to but not at the site of insult may suffer only a shortage of oxygen. Cells that are even more distant may be swept away by an over-enthusiastic immune system intent on clearing up an injured site. Anti-caspase therapy is aimed at these surrounding cell populations. Rodent models of stroke suggest that the number of dying cells can be halved by broad spectrum caspase inhibitors, which are thought to shut down both apoptosis and inflammation processes.

Idun plans to test broad-specificity caspase inhibitors for acute conditions like stroke, but these inhibitors are unlikely to work in the long-term for neurodegenerative diseases. The body needs to maintain some level of cell death as an essential part of life, to eliminate unwanted cells in the immune system and the gut. "Where you only have to give the inhibitor for a day or three I think [caspase therapy] is very promising," says Kaufmann. As to whether longer-term therapy will work, he says, "I don't know if the field is far enough along to know."

This was one reason that Elan Pharmaceuticals, plc (Dublin, Ireland) decided to get out of caspase therapy. "We never felt that caspase inhibition as a chronic therapy was reasonable," says Guriq Basi, a principal scientist at Elan.

One solution is to make specific inhibitors that only target certain caspases and therefore, perhaps, certain cell types. When Elan decided to stop caspase research two years ago, says Basi, "there were multiple [caspases] and it was difficult to specify the target." The numbers of caspases has only grown since then. Kevin Tomaselli, vice-president of science and technology at Idun, admits that "there is no human disease for which I could state with a high degree of certainty that you need to target one specific caspase. The information of which caspases to target is in the works."

Idun is making small-molecule inhibitors that are specific for particular caspases (it already has some); it can then test them in *in vitro* systems that mimic conditions like hypoxia or neurodegeneration. Such testing can indicate which caspases make good targets, but only animal testing will indicate which caspases need to be avoided because of side-effects.

Caspases work in self-amplifying cascades of cleavage and activation, so Idun must determine where caspases lie on the pathway and where the pathway reaches the point of no return. "In cytokine-induced apoptosis

Table 1

Selected companies interested in ICE, caspase or apoptosis research

Company	Progress or objectives
Vertex Pharmaceuticals, Inc.	Designed the first caspase-related drug to go into phase I clinical trials – an inhibitor of interleukin-1 converting enzyme (ICE, or caspase 1) – for inflammation. Determined ICE structure and collaborated on caspase 9 knockout mouse.
Merck & Co., Inc.	Co-discovered ICE, still interested in inflammation but also acute and chronic caspase inhibition for stroke, heart attack and neurodegeneration. Merck and Idun patents overlap.
Millennium Pharmaceuticals, Inc.	Program started recently. Have issued patent for inhibitory forms of caspase 8 that lack the protease domain.
Cytovia, Inc.	Spin-off from CoCencys, Inc. Cell-based screening system for caspase inhibitors uses flurogenic substrates that penetrate and then stay inside cells. Collaborating with Aurora Biosciences Corporation.
BASF Bioresearch Corporation	Solved ICE structure; interested in caspase inhibitors.
LXR Biotechnology Inc.	Discovered the secreted apoptosis-related protein (SARP-1) in the anti-apoptotic conditioned media of quiescent cells. Gene therapy of SARP-1 with Rhône-Poulenc Rorer Gencell.
Genta Inc.	Antisense Bcl-2 for non-Hodgkin's lymphoma.

you can completely prevent apoptosis with caspase inhibitors," says Tomaselli, for in this instance caspases are known to lie near the top of the pathway. "For other cell-death stimuli, the caspase inhibitor may be functioning far enough downstream that there has [already] been irreparable damage that the caspase inhibitors cannot reverse," he says. An example of possible damage is the release of apoptotic inducers from the mitochondria, such as cytochrome *c* and the recently identified AIF (apoptosis-inducing factor).

Vertex Pharmaceuticals, Inc. (Cambridge, Massachusetts) is the first to get a caspase-related drug into trials (Table 1); Idun should get their turn sometime in 2000. Their first target is acute exacerbations of alcoholic hepatitis, closely followed by stroke (the latter in collaboration with Novartis of Basel, Switzerland).

Killing off tumor cells

Tumor cells are often faced with a poor blood supply, an inadequate extracellular matrix, and DNA damage, and yet still they live. "Tumor cells harbor multiple genetic lesions that are chronically telling the cells to kill themselves, and the cell would be dead if it didn't upregulate survival signals," says Tomaselli. This reasoning prompted Idun to search for molecules that disrupt the interaction of Bcl-2 with its pro-apoptotic partners such as Bax. With Bcl-2 inactive, Bax can induce apoptosis.

Still, nobody can be sure that tumor cells will be preferentially susceptible to anti-Bcl-2 drugs. Kaufmann does not think this should hold Idun back. "There is no reason to think that there [should be] a therapeutic index for DNA-damaging agents, but for patients who have the right tumors those drugs work," he says. "In my inexperienced youth I would've laughed at John Reed and Idun, but I think criticizing this approach [without data] is too hasty."

The challenge for now is to come up with a small-molecule inhibitor that can disrupt a protein–protein interaction. The only known inhibitors are peptides of 14–16 amino acids. Idun has enlisted the SAR by NMR technique of Abbott Laboratories (Abbott Park, Illinois; see *Chem. Biol.* **4**, 231–232) to piece together a large inhibitor from many smaller binding molecules.

Getting proteins into cells

In 1988, two groups showed that a fragment of the HIV Tat protein could cross membranes and take attached proteins with it. This evolutionary fluke is irrelevant to HIV biology. It does not require any protein component or energy source.

Unfortunately the reproducibility of transduction was, at best, poor. "This was almost thought to be a weird artifact," says Steven Dowdy (Washington University, St. Louis, Missouri). "It never progressed to a very usable system."

Dowdy decided that everyone was doing one thing to foil the method — they were using correctly folded protein. "We took a totally blasphemous approach of using denatured proteins," says Dowdy. His "magic column," with a sudden drop from 4 M to 0 M urea, did the trick, perhaps by creating an unfolded protein that can 'thread' through membranes.

Recently, Dowdy transduced cells with a modified caspase precursor that is cleaved by HIV protease to form an active enzyme. Infected cells are killed; uninfected cells are left unharmed. The technology should work for other infectious agents that depend on proteases, like hepatitis C and malaria.

Idun licensed the technology from Washington University and is seeking a partner for the caspase application; other applications will be sub-licensed. Dowdy thinks there will be plenty of takers. "This is the tip of the iceberg of what you can do with protein therapy," he says. Until now protein therapy has been limited to proteins that act outside the cell; the inside of the cell has been the domain of the small molecules. No more. "The ramifications of this are just staggering," says Dowdy. "When we have a lab meeting and come up with yet another new application for this we just laugh — there is just so much opportunity."

William A. Wells
1095 Market Street #516, San Francisco, CA 94103-1628, USA; wells@biotext.com.