Innovations

Mapping out fat profits
Millennium Pharmaceuticals, Inc.

Exercise and diet are all very well, but for the pill-happy and increasingly obese Western world, a drug to cure the fat woes is a delicious prospect. Millennium Pharmaceuticals, Inc., of Cambridge, Massachusetts, USA, is relying on genetics and corporate partnerships in a high stakes race to capitalize on this huge market.

"The whole idea of Millennium," says Jeffrey Thomas, the Project Leader for Diabetes at Millennium, "is that now at last with the genome project and automation ... we can address common diseases. The amount of information we can bring to bear on the diseases makes the complexity more tractable." And the biggest of the complex diseases is obesity.

In the beginning there was leptin

The explosion in obesity research began in 1994, when Jeff Friedman of Rockefeller University in New York discovered leptin. Fat cells release leptin into the bloodstream, and the protein then signals to the brain that there is cellulite available, so that it decreases food intake and increases energy expenditure. The addition of leptin to normal mice makes them skinnier.

Amgen Inc. leapt on the leptin discovery in 1995, licensing the use of the protein from Rockefeller University. Millennium and Hoffman-La Roche, became involved later the same year with the discovery of the flip side of the equation, the cell-surface receptor for leptin. The Millennium/Roche team, led by Louis Tartaglia, the Director of Metabolic Diseases at Millennium, found that labeled leptin bound to the choroid plexus in mouse brains. By dissecting out this tiny organ from 300 mouse brains, the scientists were able to construct a cDNA expression library, which they then screened for leptin binding. The protein that they identified is a single membrane-spanning receptor, most closely related to the signal-transducing component of the receptor for several cytokines. The Millennium group and two groups from Rockefeller later confirmed that the receptor was the product of the mouse *diabetes* (*db*) gene. As with mice mutant for the leptin gene (*obese* or *ob*), mice mutant for *db* are fat.

Fat control in the brain

Given that weight loss leads to decreased leptin production, and thus weight gain, the next focus for research was in the brain. What happens after the leptin signal is received? Late last year, a group led by Richard Palmiter at the University of Washington at Seattle found that deleting the gene for neuropeptide Y (NPY) made *ob* mice less obese, implicating NPY in the response to starvation and lowered leptin levels.

Millennium tackled this problem using genetics and a fat, yellow mouse called *agouti*. The agouti protein is normally restricted to hair follicles, where it antagonizes the melanocortin-1 receptor and so induces a switch in pigment production. In *agouti* mice, the agouti protein is made throughout the mouse. The Millennium/Roche team and a group from the Oregon Health Sciences University led by Roger Cone showed that, in this situation, the agouti protein can also antagonize the melanocortin-4 receptor (MC4-R), thus causing obesity. Deletion of the MC4-R gene results in late-onset obesity. In contrast to NPY, the MC4-R may be involved in the response to high leptin levels and weight gain.

To market, to market

While Amgen has begun trials with leptin, Millennium has a patent for therapies that target the leptin receptor (and presumably the lawyers to defend any area of overlap with Amgen). Tartaglia is most excited by this target. "If we were to succeed [in finding] a direct leptin receptor agonist," he says, "I think we would make it into clinical trials." But he says that Millennium remains interested in the MC4-R project, because it has historically been easier to find agonists of seven-transmembrane receptors (such as the MC4-R) than single-transmembrane receptors (such as the leptin receptor).

The choice of therapeutic target is not necessarily driven by the cause of the disease. There is a disputed linkage between human obesity and mutations in the β3-adrenergic receptor, and some obese people appear to have more unbound circulating leptin, but little is known about the actual cause of obesity in the majority of humans. "In a way I think it's irrelevant," says Tartaglia. "People are going to be obese for a number of reasons, and some for a complex mixture of reasons. Our job is to identify important pathways and super-stimulate them."

Using the same reasoning, other companies are throwing the kitchen sink at obesity. Two drugs have recently been approved for marketing in the United States. Orlistat, developed by Roche Biosciences, catches the offender at the starting gate, inhibiting gastric lipase and so reducing fat absorption. Redux, or dexfenfluramine, boosts levels of the neurotransmitter serotonin, so the patient feels full (the serotonin receptor 2c regulates satiety). Both drugs have side effects: for Orlistat there are unpleasant consequences from unabsorbed fat, and Redux causes fatigue and insomnia.

Other companies are examining a handful of gut peptides that signal satiety by a variety of mechanisms, and leptin mimetics and NPY-1 antagonists are also being developed. ObeSys is even testing polyclonal antibodies to fat cells, although thus far only for the production of the perfect pork chop.

Spinning the metabolic wheels

One of Millennium's alternative approaches to obesity involves redirecting metabolic energy towards heat production. At the center of the heat-generating machinery are the uncoupling proteins, which allow protons to flow down the proton gradient across the mitochondrial inner membrane without generating ATP. UCP, the first uncoupling protein to be isolated, is unlikely to be a useful target for human therapies. It is found only in brown fat, which is common in rodents but lost by humans one year after birth.

Millennium have patented the use of a second uncoupling protein, UCPH, which they isolated based on its homology to UCP. Expression of UCPH is toxic to yeast only if the yeast are using aerobic respiration, and UCPH disrupts the proton gradient across the inner mitochondrial membrane. This latter effect was measured using a charged dye, $DiOC_6$, which inserts in the membrane only if there is a charge gradient across the membrane.

Tartaglia admits that UCPH stimulation may create problems in temperature regulation, but he believes that other temperature regulation systems will kick in, and that the increase in UCPH activity needed to burn off fat will cause a negligible increase in core body temperature.

UCPH may be an ideal drug target. Various fatty acids are known to be positive regulators of the protein's activity, says Tartaglia, so UCPH "is designed to accept positive regulation by small molecules."

The consequences of obesity: diabetes

The primary risk factors for late-onset (non-autoimmune, type II) diabetes are age and obesity. The exact nature of the connection between obesity and diabetes is unknown, but a substance produced by fat cells probably induces insulin resistance in target tissues like muscle. Initially, the pancreas keeps up by producing more insulin; later it is overwhelmed and diabetes results.

The incidence of diabetes has been rising in the Western world, mirroring the rise in obesity. But there are also genetic factors, and it is those factors that have piqued Millennium's interest. The high-throughput genotyping ability of Millennium was put to use in a recent collaborative study of an isolated Finnish population (the subjects speak Swedish, while the surrounding population is Finnish-speaking). The study identified a linkage between low insulin secretion and a region on chromosome 12. This region includes the gene that causes MODY3, a rare, dominant, early-onset form of diabetes. It is not yet clear whether MODY3, a widely expressed transcription factor, and the new gene, NIDDM2, are one and the same. The company has also announced the mapping of another, distinct gene for diabetes, although they are not disclosing the identity or location of this gene.

To track down a gene involved in a complex trait, human geneticists need a large number of variable (polymorphic) markers. Millennium most commonly uses microsattelite markers, variable simple sequence repeats like $(CA)_n$ $(n > 10)$ that are susceptible to mutation by slippage of the DNA replication apparatus. PCR amplification of the markers using surrounding simple sequence primers gives a large quantity of DNA for size comparisons. For a complex trait, 400–500 markers (one marker every 8–10 cM) are typed for each of 1000–2000 individuals.

This is no small feat, and complexities arise. "Ideally we want a box where we put DNA in one end and get a genotype out the other," says Thomas. But DNA duplications and ambiguities mean that human intervention is inevitable. Millennium and other high-throughput outfits are now looking at using point mutations as alternative markers. Although there are only two possible states (mutated or not), systems based on this hybridization technology are much more amenable to automation.

The human genome project will also help in the gene hunting. "Ultimately it's going to eliminate a step in the process," says Thomas. The initial mapping will still be necessary, but then the researchers will skip directly to the correlation of the disease with mutations in genes, without first having to define the existence of those genes.

Atherosclerosis and other matters

Cardiovascular disease is another focus for Millennium. In collaboration with Michael Gimbrone of Brigham and Women's Hospital in Boston, Millennium scientists have isolated genes that may be involved in atherosclerotic plaque development. The genes are induced by culturing endothelial cells in a flow cell designed by Gimbrone. In the flow cell, uniform shear stresses mimic blood flow typical of plaque-free areas, and turbulent stresses mimic the conditions in plaque-rich areas.

For each of the areas of research mentioned above, and several others that are less well developed, Millennium has entered into collaborative agreements for drug development. The collaborators include Hoffman-La Roche (obesity and diabetes), Eli Lilly (atherosclerosis), Wyerth Ayerst (psychiatric disorders) and Astra AB (asthma and allergies).

With these collaborations, Millennium can hand off many of the expensive and risky stages of drug discovery. But even sticking to target discovery is not foolproof. Although Millennium can estimate how much genetics contributes to a disease, it is impossible to get good estimates of how common a disease gene will be in a population, and how many fold increased risk it will confer. Fat profits, it seems, are never a sure thing.

William A Wells, Biotext Ltd,
211 Hugo Street, San Francisco,
CA 94122-2603, USA; wells@biotext.com.