Innovations

Could mitochondria be the key?

MitoKor

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As companies focus on an aging population, they are finding that it is more difficult to get a handle on the causes of disease. Where once there were defined single causes such as bacteria, there are now the variable and multitudinous risk factors for cancer and heart disease.

And then there are the degenerative diseases, in which the body has simply run out of steam. MitoKor, based in San Diego, California, is working on the assumption that it is the mitochondria that fall first, or at least hard, in some of these diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). The theory is supported by an attractive model that could explain the sporadic nature of the diseases, and by evidence of mitochondrial dysfunction. But a verdict on whether this dysfunction is real or relevant may have to wait for clinical trials of a drug that does not yet exist.

Effects of defective mitochondria

MitoKor began in 1991, as Applied Genetics. It was based on the work of W. Davis Parker, who was at that time a pediatric neurologist at the University of Colorado. "He had observed similarities in the pathology and history of diseases of the very young and very old," says Walter Moos, formerly of Chiron and now the CEO of MitoKor. "This was traced to energetic deficiencies and ultimately to mitochondria."

"A lot of people have always believed that mitochondria are so critical that almost any significant defect would be fatal," says Moos. Something close to that was happening in the rare pediatric cases — a mutation in an energy-generating pathway was causing rapid and drastic deterioration. The adult patients reached a similar point, but only at an advanced age and after many years of slow disease progression. Perhaps, thought Parker, the adult diseases were less severe versions of the pediatric diseases.

It is not surprising that mitochondria should be the first part of the cell to wear out. Respiration is a dangerous process, involving the transfer of electrons from one carrier molecule to another. The electrons' destination is molecular oxygen; in the final reaction they are used to reduce it to water. But if there is a block in the electron-transport chain, or if one component is more active than the next component, the stray electrons can damage the mitochondrion. Most often the electrons help form reactive oxygen species (ROS), which can damage proteins, DNA and membranes. Damage to the membranes can short-circuit the charge gradient needed for ATP production, and release pro-apoptotic molecules like cytochrome *c* that can trigger programmed cell death.

Explaining sporadic disease

Mitochondria as a cause of late-onset, sporadic disease makes sense. The late onset is consistent with a gradual buildup of damage, which should be at its most severe in cells with high energy requirements and little or no cell division to dilute the damage. And sporadic disease could be explained by the idiosyncrasies of mitochondrial DNA inheritance.

Although neither AD nor PD shows anything like strict Mendelian inheritance, there is some clustering of the diseases in families. For both diseases, individuals with affected mothers are at greater risk; this is consistent with maternal inheritance of a mitochondrial DNA mutation.

Any given cell of any given individual may have several different mitochondrial genomes, a condition termed heteroplasmy. There is stochastic inheritance of these different genomes in the cell divisions of both oocytes (leading to differences between siblings and even 'identical' twins) and somatic cells (leading to mosaicism and tissue-specific effects). Varying dosages of these different genomes could explain variation in the time and severity of disease onset.

The physical evidence

A theory is all very well, but a company needs hard evidence. Parker, who is now based at the University of Virginia, found in 1990 that AD patients had a defect in complex IV of the electron-transport chain; the previous year he found that PD patients had a defect in complex I. The latter defect is consistent with an animal model of PD, which relies on the use of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to inhibit NADH:ubiquinone oxidoreductase, a component of complex I. And the fact that the two disease defects are distinct, and hold true outside of the brain, give Parker and MitoKor confidence that they are not looking at a nonspecific response to cell death in the brain.

Not everyone is so confident. "I'm of an open mind," says David Clayton, of the Howard Hughes Medical Institute in Chevy Chase, Maryland. "I don't believe [the possible importance of mitochondria] has been ruled out, particularly as a secondary consequence."

Eric Shoubridge of McGill University (Montreal, Canada) is also unsure. "There's no general agreement on whether [these effects] are real or not real, or primary or secondary," he says. "If there was clearly a strong dependency [of disease on mitochondria] I think there would be strong agreement."

The clincher would be a specific mitochondrial mutation, presumably in complex IV genes. In the April 1997 *Proceedings of the National Academy of Sciences*, Parker and MitoKor reported that AD patients

had a higher incidence of five missense mutations: two in cytochrome *c* oxidase (CO) subunit I and three in CO subunit II. Any given patient had a mixture of the 'mutant' and wild-type sequence, but this could be explained by heteroplasmy.

The size of the difference was not huge — approximately 18–25% mutant sequence for AD patients, compared to approximately 12–15% for controls — but it allowed the researchers to identify 20% of the AD patients who had levels of the mutant allele exceeding those of any control case. Sixty percent of AD patients, but only 20% of control subjects, had over 20.3% mutant alleles.

The result began to unravel in the very same paper, in a note added in proof. The authors spotted their mutant alleles in databases of the wild-type mitochondrial DNA sequences of several of the great apes, raising the question of whether all great apes carry an AD mutation.

The answer came in December 1997 from Douglas Wallace (Emory University, Atlanta, Georgia) and Eric Schon (Columbia University, New York). They found that the 'mutant alleles' were pseudogenes in the nucleus; the difference between diseased and control presumably reflected decreased mitochondrial copy number in those with AD.

"There do appear to be changes in the mitochondrial DNA that track with the disease," says Moos. "It could be absolute [genome] loss, or mutations that we haven't found. It's possible that each individual has a different set of mutations."

Whatever the explanation, he says, "the test we have still associates with the disease." This primer extension assay measures the relative copy number of the mitochondrial genes and the nuclear pseudogenes (i.e., the mitochondrial DNA copy number). MitoKor sells use of the test to pharmaceutical companies who are running trials of their AD drug candidates; the test is now being used in the sixth trial of this type. "We're trying to determine if it is useful in picking responders," says Moos, "but none of the drugs in studies we have completed to date have worked well enough to find an effect."

Finding drugs

One of MitoKor's key technologies is its 'cybrid' cell lines. These lines are made by fusing diseased, anucleate cells (such as platelets from an AD patient) with normal, nucleated cells that lack mitochondria. By transferring AD mitochondria, MitoKor also transfers AD traits such as decreased cytochrome oxidase activity, increased ROS production, and increased production of β-amyloid, the protein fragment that aggregates in AD brains. MitoKor has developed a similar cellular model for PD and type II diabetes.

Before the Wallace and Schon papers, MitoKor believed it was transferring mutant complex IV genes with the AD mitochondria, but now the story appears to be more complicated. "These papers put the ball back in the company's court to explain the cybrid data, and what is being transferred," says Shoubridge.

Meanwhile, MitoKor is looking for differences in protein expression between cybrids with normal and AD mitochondria. These differences could be exploited in diagnostic tests, or the proteins could be future drug targets.

But Moos's main interest is in using the cybrids as cellular models of disease, for finding and testing drug candidates. "It's clear that the activity level of complex IV is decreased, perhaps because of an incorrect stoichiometry [only three of the thirteen subunits are encoded in mitochondria]. So we've focused on the ultimate level of function."

That ultimate readout is the status of the mitochondrial permeability transition pore (PTP). The PTP is defined more by its electrical behavior than its molecular composition: when it opens it releases ions and metabolites and the respiratory chain collapses. MitoKor's task is to improve mitochondrial function and thus forestall the opening of the PTP.

"We've found a number of targets that influence the [mitochondrial permeability] transition or apoptosis," says Moos. "We don't typically go after the electron-transport chain itself, as we would have to improve enzyme activity." But MitoKor is testing some defined (but undisclosed) protein targets, in addition to doing whole-cell assays. Animal testing of candidate molecules has been started.

Are mitochondria the answer?

"Alzheimer's disease, like many diseases, is not one disorder, it's several," says Moos. "People will agree or disagree [with the importance of mitochondria] based on their own personal research because this is not one disease. I would characterize it as at least three different disorders which all present with the same symptoms."

MitoKor does not assert that their mitochondrial theory will explain all AD and PD; their estimate is closer to 50%. Moos says the same may be true for type II diabetes. "As with Alzheimer's disease, I'm not sure that anyone has this all together yet, other than their particular bias."

"One of the things I've been amazed about is how complicated these diseases really are and how much difference of opinion there is as to what is primary versus secondary," he says. "It's far from clear what the primary events are. And it's not terribly relevant whether mitochondria are primary or secondary, if you can treat the disease by treating the mitochondrial defect."

MitoKor would be happy to link a mitochondrial mutation to AD, but Moos says it is not essential. "It's easiest to sell the story if you have the mechanism all the way back to the genetics," he says. "But there are certainly a lot of drugs that have made it to market with an unknown mechanism. At the end of the day the big ticket is the drug."

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