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EVENTS

Seattle Conference
Biotech Anti-Aging Companies Convene

In one of the most comprehensive conferences on anti-aging research, new insights across the life sciences revealed novel approaches to understanding and intervening in disease and other conditions of human aging.

By Pamela Tames

In the first conference of its kind, scientific researchers and representatives of biotech companies met in Seattle, Wash., last summer to present new findings on the molecular mechanisms and genetics of aging and age-related diseases.



Aging is complex and, as was made clear at the conference, there's no one theory that "explains it all." However, scientists now have good models and tools. Many agree that genomic studies—the study of gene expression and function—is an important pathway to follow. Already it has opened up some promising lines of inquiry, giving many reasons for optimism. For example:

Some of the genes giving rise to longevity ("gerontogenes") have been isolated and characterized. Researchers believe they number in the "handful," not hundreds.

Drosophila (fruit fly) studies indicate no maximum limit to life span.

Evolutionary biology, which describes how natural selection affects which genes are passed on, is yielding clues to aging processes and candidate genes for drug

discovery.

- In model systems, long-lived mutants are more resistant to oxidative woes (from free radicals) and other stresses, confirming a causative role for oxidative stress in aging.

Looking for DNA sequences that code for aging is the "new gold rush," says George M. Martin, professor of pathology at the Alzheimer's Disease Research Center, University of Washington, Seattle. Martin described how concepts in evolutionary biology have led him to characterize the period when we show obvious physical signs of aging as a distinct "sageing phenotype."

Evolutionary biology theory says that the force of natural selection is directed at maximizing survival and reproductive capacity of an organism. It thus favors beneficial alleles (genes) and disfavors deleterious ones. However, the power of natural selection wanes with age, so early (reproductive) benefits are under powerful selection, while later detriments are under weak selection.

That means there's a "tradeoff," Martin explained. You get the good with the bad. Mutations and gene actions that were initially selected for because they make you more fit reproductively, paradoxically cause age-related effects later in life. And because the force of natural selection has weakened so much by then, "you can't escape them."

Martin says this indicates that aging evolved in a "non-adaptive way." That is, senescence is a phenotype that has escaped the force of natural selection, so there's "a lot of plasticity of life span," explains Martin.

Michael Jazwinski, professor, departments of biochemistry and molecular biology, Louisiana State University Medical Center, discussed his studies of longevity genes in yeast, studies that have led to the identification of mechanisms related to increased life span and stress-response. He believes these may be conserved across species and thus, are relevant to humans.

Jazwinski believes his research highlights the importance of genetic and both internal (hormonal) and external environmental factors in aging. While metabolic capacity and stress response are genetically determined functional potentials, they are also modulated by environmental factors through damage, stress and disease.

There is obvious relevance to humans, say Jazwinski. "We need to find ways to expand our metabolic capacity to improve behavior

and function later in life. In order to do that we can manipulate certain genes, but there are limits in which we can operate." Exercise is a good example of those limits: just enough and the muscle fibers repair and get stronger; too much and they're damaged. This principle of limits, says Jazwinski, "applies to both physical and psychological stresses."

A lot of research has been done on Alzheimer's disease. But, says Gerard D. Schellenberg, associate director for research, veterans affairs, Puget Sound Health Care System, in Seattle, "A big part of the picture is still missing in terms of our understanding of the underlying mechanism. It might be oxidative damage."

Four million people in the U.S. are affected by Alzheimer's disease; their care costs about \$90 billion to \$100 billion a year. It's known that Alzheimer's disease is a neurodegenerative disease affecting the central nervous system exclusively. Its main features are: formation of B-amyloid plaques, neurofibrillary tangles, synaptic loss, and neuronal atrophy and neuronal death.

Attacking Alzheimer's at The Root

According to Schellenberg, finding good animal models with B-amyloid plaques has been a major achievement. He has been using mice to look for genes modifying Alzheimer's disease characteristics. So far, he's found a few important ones (PS1, PS2, and ApoE) and confirmed that B-amyloid is the critical molecule in all forms of Alzheimer's disease. "But these genes don't explain all the variations and features seen in Alzheimer's disease," says Schellenberg, "so we're missing some genetic risk factor. Whether it's related to aging or not, we don't know."

This year, Bryant Villeponteau, vice president of research, Jevone Pharmaceuticals, and three colleagues won the prestigious "Distinguished Inventors of the Year" award for their telomerase patent. Telomerase is an enzyme that maintains chromosome caps (called telomeres), believed to be the basis of cell "immortality." Now he's onto a new model-the heterochromatin loss model-which he thinks could potentially explain more about aging.

Villeponteau's starting point for biotech research at Jevone is the premise that aging has multiple causes, one being oxidative damage. Oxidative damage refers to the accumulated damage to DNA and/or proteins through free radicals, or reactive oxygen intermediates (ROIs). Based on recent data, ROIs have been linked to many degenerative diseases.

ROIs may also play a role in Villeponteau's heterochromatin loss model, the focus of Jevone's research effort. Heterochromatin loss describes structural changes in the chromosomal material. During embryonic development, so-called heterochromatin domains are laid down in clumps, effectively "silencing" the expression of certain genes. Because only certain genes are allowed to "talk," the cell becomes differentiated into specific tissues.

Where aging gets tied into this picture is during cell division: every time a cell divides, heterochromatin wanes, and there's a net loss. "The implication is that heterochromatin loss is a clock of cell senescence," says Villeponteau. And, there's evidence that ROIs may play a role in accelerated waning of heterochromatin, he says.

Attention also was spent on the life span of fruit flies, and the lessons they may impart. Using an approach known as QTL (quantitative trait loci) mapping and about 700,000 *Drosophila* flies, Jim Curtsinger, professor, ecology and behavior, at the University of Minnesota, has found evidence reversing a long-held idea about maximum life span. Specifically, there is none.

Traditionally, it's been assumed that life span is fixed. "Almost everyone," says Curtsinger, "will make it to 85, give or take seven years, and then almost everyone will check out." Working from Scandinavian church records, the most accurate human longevity data he could find, Curtsinger found two patterns: a systematically increasing maximum life span and a decreasing mortality rate. Using techniques of gene manipulation, he recreated these patterns in *Drosophila*, producing flies that lived twice as long as controls. "I'm not saying the flies are immortal," explains Curtsinger, "They wake up every morning and play Russian roulette- their probability of dying is about 20 percent. What's important is the probability of death is not increasing with increasing age." In other words, there's no "preordained maximum."

Moving on to a different sort of animal, Thomas E. Johnson, professor, institute of behavioral genetics, at University of Colorado, discussed *C. elegans*, a nematode (worm) species that has been widely used to study aging. A number of longevity genes have been found in this creature. Johnson's studies, for example, have produced long-lived nematode strains in which a single gene mutation results in a 65-percent increase in life expectancy and 110-percent increase in maximum life span.

The fact is, "a whole complex of genes in nematode can increase life span," says Johnson. "We're hoping to identify candidate genes in nematode first and then test these for prolongevity in humans."

A key feature of all these long-lived mutants, says Johnson, is exceptional resistance to environmental stresses such as heat, oxidative damage and ultraviolet exposure. In addition, the mutants appear healthier, as evidenced by greater motion at all ages and a higher metabolic capacity. "It appears that this class of gerontogenes," says Johnson, "regulates at a molecular level

(possibly mediated by increased expression of the antioxidant enzymes, SOD and catalase) multiple stress-response pathways as well as longevity." According to this stress-response hypothesis, then, genetic and non-genetic interventions that increase an organism's ability to respond to stress may prolong life. So far, the only comparable intervention in animal models, says Johnson, is dietary restriction, which has been reliably demonstrated to increase life span. He believes the underlying mechanism here may also be increased resistance to acute environmental stress. If that's the case, it may well be "we've been overly cautious about trying to minimize our exposures to things that, while hazardous at very high levels, may well be beneficial at lower exposures," says Johnson. Oxidative stress may also play a role in Alzheimer's disease. In addition, aging and Alzheimer's disease are often a linked discussion. A feature of Alzheimer's is the presence in certain brain regions of senile plaques, entities composed of B-amyloid. Using cultured neurons *in vivo*, Allan Butterfield, professor of chemistry and director, Center of Membrane Sciences, at the University of Kentucky, has investigated the role of B-amyloid in causing Alzheimer's disease. He's shown that B-amyloid plaques in Alzheimer's disease are areas of elevated oxidative stress, generating free radicals that destroy neuronal membrane components.

Dangerous Free Radicals

"This new finding," says Butterfield, "helps explain how one disease can have so many things go wrong."

Using a probing technique called spintrapping, Butterfield observed that B-amyloid produces free radicals, which, in turn, cause lipid (fat) peroxidation. Lipid peroxidation damages membrane lipids, proteins and nucleic acids. A by-product of this process is a very toxic molecule known as 4-hydroxy-2-trans-nonenal, or HNE, which also destroys membrane proteins, says Butterfield. The net effect of all these changes is cell death.

In studies, adding antioxidants such as glutathione and vitamin E protected neurons from HNE damage and B-amyloid-induced lipid peroxidation. This may explain findings recently reported in *The New England Journal of Medicine* in which high daily doses (2,000 IU) of vitamin E kept people with Alzheimer's disease out of nursing homes by at least six to seven months, explains Butterfield.

Butterfield believes it's possible to develop more powerful antioxidants than vitamin E. He's consultant for a company, Centaur Pharmaceutical, that's trying to do just that by modifying the basic structure of the non-therapeutic molecule, N-tert-butyl-alpha-phenylnitron (PBN). In experiments, this brain-accessible free radical scavenger has extended mean life span in mice by 50 percent.

Any discussion of aging these days involves the biomarkers, or scientific measures, of aging itself. The National Institute on Aging is now in the 9th year of a 10-year mice/rat study that is seeking to establish the measures of biomarkers. Findings from this study will next be tested in humans.

Biomarkers will allow researchers to objectively measure rates of aging in all systems, such as cardiovascular, behavioral, neuroendocrine, immunologic, and others, as well as results from interventions. An important criteria for all biomarkers is that they are convenient and minimally invasive for humans.

Aging is an immensely complicated topic, perhaps even more so than AIDS. "Aging," says Calvin B. Harley, chief scientific officer at San Francisco-based Geron Corp., "is very complex. There are a multitude of effects." One way around that is to focus on a single aspect of the process, such as the difference between normal and senescent cells.

At Geron, researchers believe the difference lies in the telomeres, those DNA sequences that cap the ends of chromosomes and may function as cellular clocks. Harley discussed how Geron is applying the telomere model to developing diagnostics and treatments for such age-related diseases as cancer and atherosclerosis.

The Telomere Theory

Every time a normal (somatic) cell divides, the telomeres shorten a bit. When the telomeres have shortened to a critical point, gene expression changes: the cell stops replicating and becomes senescent. Anything that increases cell turnover—a chronic localized stress, for example—will accelerate cell replication, driving the cell into earlier senescence.

Harley believes the altered pattern of gene expression, which marks senescence, may underlie reduced tissue regeneration and ultimately age-related disease. "We are using these genes as markers of the aging phenotype," says Harley, "and targeting anti-aging therapies at the upregulated or downregulated enzymes."

Another application of the telomere model to life extension includes finding ways to stabilize the telomeres themselves. Harley believes it may be possible to do this by increasing the activity of the telomerase enzyme, which (as shown by Jevone Pharmaceuticals) appears to lengthen telomeres. Geron is cloning telomerase components in order to understand its regulation.

The flip side of this-telomerase inhibition-also has an application. Cancer cells show high telomerase activity. "Inhibiting telomerase might kill, or 'mortalize,' tumor cells," says Harley. Based on the finding that telomerase expression can be detected in a majority of tumors and this correlates to clinical outcome, Geron just launched TeloQuant, a proprietary telomere length measurement assay.

Telomere length may also serve as a potential marker of replicative aging in human vascular (endothelial) cells. Geron has shown that endothelial cells lose telomeres as they replicate. The accumulation of senescent endothelial cells in arteries may contribute to atherosclerotic plaque formation and thrombosis (excessive blood clotting). A similar process may also explain dermal (skin) atrophy.

Geron's most recent research program involves creating new tissues from primordial stem cells, unique germ-line cells that are undifferentiated and immortal (that is, show telomerase activity). Through gene manipulation, these cells can be differentiated into any and all types of cells and tissues and then transplanted in the body. Geron is using this technology to differentiate primordial cells into cardiomyocytes (heart muscle cells) for treatment of congestive heart failure, and neurons for treatment of Parkinson's disease.

Another company, Seattle-based LifeSpan BioSciences, Inc., is applying its proprietary gene discovery technology to identify the top 1 percent of therapeutic and diagnostic targets among the 100,000 genes in the human genome. "The technology," says Glenna C. Burmer, chief scientific officer at LifeSpan, "is designed to discover disease-associated genes and proteins, determine their expression, localization and function, and then identify the best targets for drug discovery."

The traditional way of discovering drugs is to test thousands of compounds from plant extracts. That takes 10 to 15 years and costs about \$100 million to \$300 million. "In the past five years," says Burmer, "the genomic revolution has changed the paradigm of drug discovery." An example of the success of this approach is the discovery of the protease inhibitors used in AIDS.

But finding ideal target genes is not easy. In the public domain databases, only about 10 percent of genome (10,000 genes) are fully sequenced, and only about 5 percent of those are associated with disease. So LifeSpan had to create its own gene source database. They now have a tissue bank of 100,000 human samples, taken at every decade of life, representing 175 organs and 500 diagnostic disease categories.

According to Burmer, LifeSpan is "basically a gene discovery and target validation company," and doesn't plan to develop drugs. "There's a huge opportunity in just looking for new drug targets in the human genome and many of these will lead to new drugs for aging diseases and consequently, aging in general," she says.

Death-Defying Neurons

At Cephalon Inc., researchers use neurodegenerative diseases (such as Alzheimer's disease, Amyotrophic Lateral Sclerosis (Lou Gehrig's disease), and Parkinson's as models for aging. The premise here, explains John Farah Jr., director of scientific affairs at Cephalon, "is if we can spare neurons at risk of dying in neurodegenerative diseases, maybe we can also do the same for aging." To this end, Cephalon is developing small molecule drug candidates that target intracellular kinases, proteases and transcriptional regulators.

Researchers have created rodent models with neurodegeneration leading to impaired cognition. Using an orally administered compound (CEP-3265, a vitamin D analog that has none of vitamin D's toxic effects), they showed it could reverse neuronal atrophy and may also be protective. CEP-3265 appears to work by causing expression of growth factor genes in the central nervous system.

In other studies, they have used another small molecule, CEP-1347 (a derivative of a natural product), to selectively inhibit kinases. Kinases block stress/injury-activated signaling, leading to altered gene regulation and cell death. CEP-1347 appears to promote survival of neurons affected with a variety of lesions.

Eventually Cephalon hopes to test these small molecules in people with Alzheimer's disease. "Even if we don't cure Alzheimer's disease, we may make a significant impact on disease progression," says Farah.

Another company presenting at the conference was Aquila Biopharmaceuticals. "Our program," explains Richard T. Coughlin, senior director of microbial disease research at Coughlin, "is to fight disease in advanced years through immune modulation. We're talking about vaccines." He's directing clinical trials through the National Institutes for Health, with the goal of producing a second generation pneumococcal vaccine for use in the elderly.

With age, we become more susceptible to infectious diseases and they cause greater morbidity and mortality. Pneumococcal infection, in particular, is a major cause of death in the elderly. The problem is just when we need vaccines the most, they're least

effective. Even the newer conjugate vaccines are only effective in 50 to 60 percent of the elderly and there are side effects and resistance problems.

Aquila researchers believe they have overcome these obstacles by addition of the saponin adjuvant, QS-21, to the pneumococcal vaccine. In studies, QS-21 "raised a very potent antibody response, more so than any other adjuvant," says Coughlin.

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