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Innovations
FROM IDEA TO IND

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PRODUCT R&D

RAISING THE COHBAR

By Lauren Martz, Staff Writer

A recently discovered mitochondrial peptide with metabolic effects on skeletal muscle suggests mitochondrial DNA may deserve a second look as a source of therapeutic targets. A group from CohBar Inc. and the University of Southern California found the peptide encoded in the mitochondrial genome, showed it mimics the effect of exercise and believes it could help treat Type II diabetes.

It's far from clear how many therapeutic targets might be encoded in mitochondrial DNA, although that has been the basis of CohBar's platform since it was founded in 2007. In January, the company completed an IPO that brought in $11.25 million to help develop the discoveries within the company's labs.

Although the first therapeutic peptide from mitochondrial DNA — humanin — was discovered by CohBar's founders and other groups over a decade ago, no other new targets have been published until now. Humanin is secreted by mitochondria and protects cells from apoptosis and oxidative stress, and CohBar is developing the peptide and its analogs for Alzheimer's disease (AD) and other indications.

The discovery of humanin contradicted the long-standing idea that mitochondrial DNA contains only 37 genes, encoding 13 proteins, that act inside mitochondria. Those proteins are considered poor drug targets because delivering therapeutics across the multiple membranes that surround mitochondria is difficult.

In addition, humanin's discovery challenged the widespread belief that mitochondria — which create energy and regulate the response to oxidative stress — receive cellular signals but do not send signals to the rest of the cell.

"Humanin opened up the possibility that there might be additional biologically active peptides encoded in the mitochondria," said CohBar co-founder Pinchas Cohen. "We know that the mitochondria need to communicate with the rest of the cell and the rest of the body."

He added there could be as many as 80 unidentified short bioactive peptides encoded in the mitochondrial genome.

Cohen is dean of the USC Davis School of Gerontology and executive director of USC's Ethel Percy Andrus Gerontology Center.

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Now, through its USC collaboration, CohBar has identified a second mitochondrial peptide, MOTS-c, that is secreted by mitochondria and shows activity in preclinical models of obesity and diabetes. (See "MOTS-c translation", page 6). Cohen told BioCentury the team has also found several other mitochondrial peptides that it has not yet disclosed, including a family of six small humanin-like peptides (SHLPs).

MITOCHONDRIAL MUSCLE

Cohen and colleagues identified MOTS-c by using a computational model to search for new short open reading frames within the mitochondrial genome that would represent proteins when translated using either the mitochondrial or standard genetic code. The compound is a 16-mer peptide encoded within the mitochondria's 12S rRNA.

But the unexpected finding was that the peptide could not be translated within the mitochondria by the organelle's translation machinery because the sequence contains mitochondrial stop codons. That meant the rRNA has to be transported out of the organelle for translation by the cytosolic machinery — in contrast to the 13 known mitochondrial proteins which are translated inside the organelles.

In humans and in mice, MOTS-c was present in circulating blood and skeletal muscle. To confirm the peptide was produced exclusively by mitochondria, the team depleted mitochondrial DNA from HeLa cells and found MOTS-c and its transcripts were eliminated.

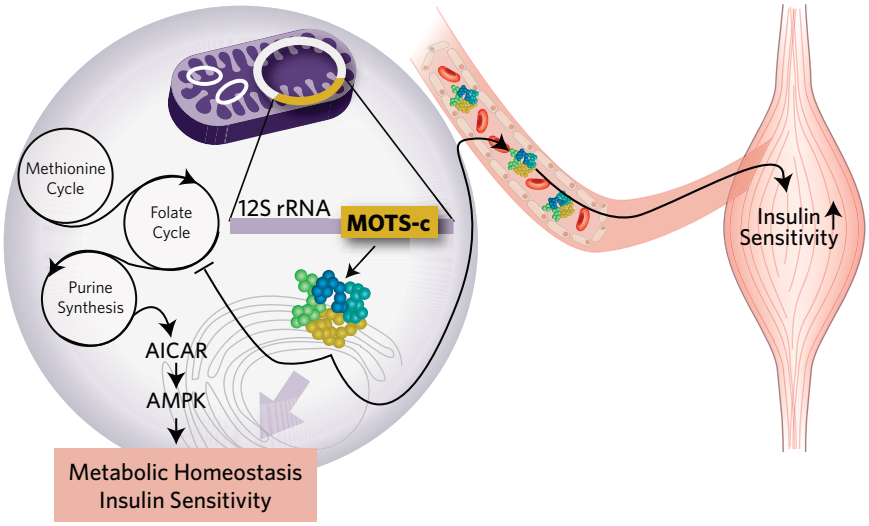
MOTS-C TRANSLATION

A group from the University of Southern California has identified a new, short mitochondria-derived peptide, MOTS-c, that is encoded within the mitochondrial genome and promotes metabolic function.

MOTS-c is transcribed from the mitochondrial chromosome and originates from the 12S rRNA locus. The MOTS-c transcript is transported from the mitochondrion to the cytoplasm, where it can be translated into a 16-mer peptide. The MOTS-c transcript encodes mitochondrial stop codons, preventing translation inside the organelle.

Within the cell, MOTS-c inhibits the folate cycle and its downstream purine synthesis pathway, resulting in AMPK activation. AMPK activation contributes to fatty acid oxidation, glucose uptake and insulin sensitivity. The USC team found the MOTS-c peptide in circulating plasma and skeletal muscle, suggesting it is secreted from the cell and acts at the muscle. MOTS-c stimulated glucose disposal and enhanced insulin sensitivity in skeletal muscles of mice. *Source: Based on an image from Lee, C., et al. "The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance." Cell Metabolism (2015), with permission from CohBar Inc. and the USC authors.*

AMPK - AMP-activated protein kinase; MOTS-c - Mitochondrial open reading frame of the 12S rRNA-c



Next, the researchers looked for biological activity. In human embryonic kidney cells, MOTS-c or a synthetic analog increased expression of genes involved in cellular metabolism and inflammation. Specifically, MOTS-c activated AMPK-stimulated glucose clearance.

In mice fed a high-fat diet, MOTS-c prevented obesity without decreasing food intake and decreased hyperinsulinemia and hepatic lipid accumulation compared with vehicle. Moreover, daily treatment with MOTS-c increased respiratory exchange and glucose utilization in the skeletal muscles, resembling the effects of diet or exercise.

"We found that MOTS-c actually stimulated the same pathways in skeletal muscle that are stimulated by exercise in the animals," said Cohen. "We believe it mimics exercise's effects. Mice fed a high-fat diet could continue to eat without gaining any weight."

Finally, the team looked for age-related variations in peptide levels, because mutations and damage to mitochondrial genes over time can contribute to metabolic dysfunction and other diseases that occur with age. That is in part due to the fact that mitochondrial DNA lacks the same DNA repair mechanisms that protect nuclear DNA.

Cohen's group found endogenous levels of MOTS-c were lower in aged mice than in younger mice. However, treatment of aged mice with MOTS-c restored insulin sensitivity to levels found in young mice.

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> Pinchas Cohen, University of Southern California

According to the researchers, the data suggest that restoring levels of MOTS-c could prevent or reverse age-related metabolic dysfunction and disorders.

Cohen told BioCentury the team is creating transgenic mice that overexpress MOTS-c to study the peptide's effects on aging and disease. "We already know that the levels fall with aging and that conditions such as diabetes and obesity occur with aging, but we want to see if there is a specific loss of the peptide in metabolic diseases that can be corrected by treatment," he said.

Results were published in *Cell Metabolism*. (See "Distillery", page 17)

PEPTIDE PATH

Cohen believes MOTS-c has several advantages over the many other compounds in development for diabetes and obesity.

The first is that MOTS-c treatment would involve replenishing a naturally occurring peptide whose falling levels contribute to disease pathology.

Another advantage for MOTS-c, he said, is its mechanism of action. "Other drugs for diabetes and obesity work at various sites including the liver, pancreas, gut and brain. MOTS-c is a unique hormone because it acts primarily on muscle, which is an important site of glucose utilization."

CohBar is the only company to disclose a platform for developing mitochondrial-derived peptides.

CEO Jon Stern told BioCentury the company has selected MOTS-c as its lead program and expects to conduct 12-18 months of IND-enabling studies before entering the clinic, but said the company hasn't yet selected a lead indication.

The first order of business will be to develop more potent, longer-acting analogs of MOTS-c and test them in additional animal models, Stern said.

Stern added that the company is reserving some funds for new peptide discovery and for pursuing the SHLP family that was also identified by Cohen's group.

Five of the six SHLP peptides promote cell survival and inhibit the effects of reactive oxygen species (ROS), which suggests those peptides could be active in metabolic or neurologic diseases. The sixth peptide has opposite effects — inducing apoptosis and inhibiting angiogenesis — and is being considered as a cancer therapeutic.

For now, CohBar is keeping its mitochondrial peptide assets in-house, but the company will consider partnerships for development in the future.

The University of California Los Angeles has filed patent applications covering the use of MOTS-c and its analogs in metabolic diseases. CohBar also has IP related to humanin analogs, the SHLP peptides and other undisclosed mitochondria-derived peptides. The university has exclusively licensed all IP related to mitochondria-derived peptides to CohBar.

COMPANIES AND INSTITUTIONS MENTIONED

CohBar Inc., Los Angeles, Calif.
University of California Los Angeles, Los Angeles, Calif.
University of Southern California, Los Angeles, Calif.

TARGETS AND COMPOUNDS

AMPK - AMP-activated protein kinase
Humanin (MT-RNR2) - Mitochondrially encoded 16S RNA
MOTS-c - Mitochondrial open reading frame of the 12S rRNA-c

REFERENCES

Lee, C., et al. "The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance." *Cell Metabolism* **(2015)**

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