found that both CIAP1 cleavage and apoptosis were blocked. They obtained similar results using primary mouse thymocytes — thymocytes with no p53 genes failed to cleave CIAP and failed to undergo apoptosis.

To identify the protease involved, Levine and colleagues went back to the literature. Previous reports had shown that mammalian IAPs can interact with a serine protease called HTRA2/OMI, so the authors did a northern blot analysis of *HTRA2* messenger RNA levels in HeLa cells during treatment with etoposide. They observed a seven-fold increase in *HTRA2* mRNA levels; a similar increase was also seen when HeLa cells were transfected with a p53 expression vector.

The authors therefore conclude that a parallel pathway to that first mapped out in flies indeed exists in mammals, with the subtle difference that CIAP1 is destroyed by protease-mediated cleavage rather than being targeted by ubiquitin for destruction.

Alison Mitchell

References and links

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MITOCHONDRIAL BIOGENESIS

NO energy

Mitochondria in brown adipocyte tissue (BAT) are larger and more numerous than in other cell types; their inner mitochondrial membrane contains uncoupling protein 1 (UCP1), which diverts energy from ATP synthesis to thermogenesis. Nitric oxide (NO) is known to regulate biological functions in mature brown adipocytes, but, until now, NO's role in mitochondrial biogenesis has not been studied.

Reporting in *Science*, Nisoli and colleagues looked at mitochondrial biogenesis in primary cultures of mouse brown adipocyte precursors. Treatment with an NO donor increased the mtDNA content above levels seen in untreated cells, which were due to spontaneous differentiation of the adipocyte precursors. This increase was abolished in the presence of the NO scavenger oxyhaemoglobin, indicating that it was mediated by NO generation.

The authors next showed that this effect occurred through activation of the peroxisome proliferation-activated receptor and co-activator 1α (PGC-1α) — a principal regulator of mitochondrial biogenesis in BAT, and cardiac and skeletal muscle. Using a cyclic GMP analogue and a guanylate-cyclase inhibitor, they also showed that the biogenesis depends on cGMP. And study of mouse white-fat 3T3-L1 and human monocytic U937 cell lines revealed that the biogenesis was not restricted to brown adipocytes and their differentiation processes.

To investigate the role of endogenous NO, the authors stably transfected HeLa cells with endothelial nitric oxide synthase (eNOS) — the only isoform that is expressed in brown adipocytes and 3T3-L1 cells under experimental conditions. Induction of eNOS increased mitochondrial biogenesis; an effect that was abolished by a NOS inhibitor.

Cold exposure triggers PGC-1α expression through activation of β_3-adrenergic receptors and increases in intracellular cAMP and Ca^{2+}, all of which stimulate NO production in brown adipocytes. So Nisoli *et al.* studied BAT functions in wild-type and *eNOS*$^{-/-}$ mice before and after cold exposure. At both temperatures, histological analysis indicated that *eNOS*$^{-/-}$ BAT was functionally inactive, and mitochondrial biogenesis was impaired.

When the authors looked at the control of biogenesis in the brain, liver and heart of the knockout mice, they found that deletion of *eNOS* was enough to reduce the number of mitochondria even in tissues that have a basal

level of neuronal, and possibly inducible, NOS expression.

In *eNOS*$^{-/-}$ mice, oxygen consumption rates — an indicator of metabolic rate — were decreased, indicating that BAT-dependent thermogenesis might be impaired. In genetic models of obesity, defective energy expenditure is involved in increased food intake and body-weight gain; eight-week-old *eNOS*$^{-/-}$ mice showed similar food consumption but weighed more than wild-type mice. So, the increased body weight of *eNOS*$^{-/-}$ mice could be accounted for by higher feed efficiency (weight gain/food intake) caused by defective energy expenditure.

So what does this mean? The features shown by *eNOS*$^{-/-}$ mice — reduced mitochondrial number and energy expenditure, weight gain, insulin resistance and hypertension — are all typical of the so-called metabolic syndrome. Millions of people are estimated to have metabolic syndrome, placing them at an increased risk of developing diabetes and cardiovascular disease. However, if the results reported by Nisoli and colleagues are applicable to humans, then we will have "…clues for the prevention or treatment of this condition".

Natalie Wilson

References and links

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WEB SITE

Center for Study and Research on Obesity:
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