

omes in an attempt to achieve this goal for different cell types.

An equally attractive alternative to 'building your own' is to 'browse the catalog' of naturally available AAVs, in other words identifying AAVs that exhibit desired properties of high-affinity receptor binding, intracellular transportation, expression and evasion of the natural immune response.

The new results from Wilson's group demonstrate an effective method for identifying novel AAVs with different capsid variations. New AAVs potentially have

different intracellular properties and provoke different types of immune responses. Thus, there is now real hope that naturally occurring AAVs can be identified that will transfer genes to target cells and allow them to be expressed for long enough and at high enough levels to be an effective genetic treatment. ■

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## Obesity gene therapy

# Slimming immature rats

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Gene Therapy (2003) **10**, 196–197. doi:10.1038/sj.gt.3301920

**T**he worldwide obesity epidemic has grave consequences because of increased risk of diabetes, cardiovascular disease, cancer, and other complications and reduced lifespan.<sup>1</sup> Diet and exercise are the cornerstones of treatment, but an increasing number of patients will require therapeutic intervention to decrease and maintain body weight. Now a new *in vivo* work by Satya Kalra's group at the University of Florida<sup>2</sup> shows that a gene therapy strategy has the potential to be tremendously effective as an obesity treatment in children.

To develop treatments for obesity, studies that help us understand the pathophysiology of body weight regulation are vital.<sup>3</sup> Such studies have shown that fat, rather than merely storing excess energy, also secretes substances that are actively involved in energy homeostasis as well as the complications of obesity.<sup>3</sup>

Leptin is the best known of these substances.<sup>4</sup> This hormone is secreted in proportion to body fat and regulates appetite and energy expenditure, mainly by influencing the brain.<sup>3–5</sup> Mutations of leptin or leptin receptor genes lead to overeating, impaired thermoregulation, massive weight gain, insulin resistance, diabetes, immune dysfunction, failure of sexual maturation and a variety of neuroendocrine abnormalities in rodents and humans.<sup>3–5</sup> Conversely, recombinant leptin reverses these abnormalities in leptin deficient animals.<sup>3,4</sup> Leptin has also been implicated in reproduction, angiogenesis, bone formation, brain development and regulation of the cardiovascular system.<sup>3,4</sup> These diverse effects appear to occur mainly through the long leptin receptor and JAK-STAT signal transduction pathway.<sup>3,4</sup>

The discovery of leptin created enormous excitement: surely here was a simple way of treating obesity. However, it turned out that normal animals are relatively insensitive to leptin.<sup>3,4</sup> In fact, 'common' (diet-induced) obesity is typically associated with high circulating leptin and diminished sensitivity

to peripheral leptin administration.<sup>3</sup> Reduced transport of leptin to the brain and inhibition of leptin signal transduction are both possible causes of this reduction in sensitivity.<sup>3</sup> Regardless, we still do not know if reduced leptin sensitivity is a cause or a consequence of obesity in most humans.

Gene therapy has been used to deliver leptin in genetically obese and normal rodents.<sup>6–8</sup> Adeno-associated viruses (AAV) are ideal vehicles for leptin gene therapy as they are nonpathogenic, capable of infecting nondividing as well as dividing cells, and express the transgene over long periods.<sup>7,8</sup> Using this technology, Karla and co-workers<sup>8</sup> have previously demonstrated a prolonged reduction in body weight after injection of recombinant AAV virus encoding leptin (rAAV-leptin) in the brain (central leptin gene therapy). Presumably, central leptin gene therapy circumvents leptin resistance through a paracrine or autocrine process.<sup>8</sup>

In the new study published in *Paediatric Research*,<sup>2</sup> a single injection of rAAV-leptin into the cerebral ventricle of immature rats prevented weight gain during the 10-month duration of the experiment. The treatment reduced food consumption as well as serum leptin, insulin and fatty acids, but increased uncoupling protein (UCP)-1 in brown adipose tissue (BAT) and ghrelin. The changes in BAT UCP-1 and ghrelin were observed in younger but not older animals. The authors analysed mRNA levels of neuropeptides in the hypothalamus to understand the central actions of rAAV-leptin. NPY was decreased while proopiomelanocortin (precursor of  $\alpha$ -MSH) was increased, suggesting that the reduction in appetite and body weight was mediated at least in part through hypothalamic neuropeptides. AGRP, a well-known leptin target that is colocalized in the arcuate nucleus with NPY, was not affected by central rAAV-leptin. Moreover, leptin gene therapy did not alter the timing of sexual maturation (vaginal opening) and duration of estrus cycles.<sup>2</sup>

These new data clearly show that single injection of rAAV-leptin can achieve sustained weight reduction. Moreover, they demonstrate that this strategy can be used on immature animals without harming sexual maturation or reproductive cyclicity.<sup>2</sup>

However, the mechanisms responsible for age-related differences in the response to rAAV-leptin, also found in other studies,<sup>9,10</sup> need to be investigated. For example: why does rAAV-leptin have only a prolonged effect on food intake in younger animals, but not older animals?<sup>2,8</sup> It seems that much of the long-term reduction in body weight is because of increased metabolic rate, although the effect of leptin is diminished in older animals.<sup>9</sup> In the latter case, activation of STAT-3 is normal despite the reduced physiologic response to central rAAV-leptin, suggesting that age-related leptin resistance occurs through a mechanism downstream of leptin receptors and JAK-STAT pathway.<sup>10</sup>

Unfortunately, for a number of reasons, it is unlikely that these encouraging results are immediately applicable to humans. First, intrathecal administration of rAAV-leptin is not a practical mode of treatment in large populations. Second, while it has been reported that leptin is synthesized *de novo* in the brain,<sup>11</sup> the long-term consequences of central rAAV-leptin on brain structure and function are not known. Third, the irreversibility of the rAAV-leptin and other gene therapy approaches raises safety and toxicity concerns.<sup>2,6–10</sup> There are no in-built controls for expression of the rAAV-leptin transgene, so in some circumstances (eg after continuous leptin infusion<sup>12</sup>) exposure to high leptin level can cause excessive weight loss with dire consequences in the long term. Theoretically, this obstacle may be overcome by placing the leptin gene under the control of a promoter responsive to signals involved in leptin regulation. Unfortunately, our understanding of how leptin is regulated in the brain is at best rudimentary.

Despite these issues, the Kalra's new work is an important additional step towards the development of novel therapies for obesity. ■

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