Fundamental Research on Suncus (*Suncus murinus*) as an Animal Model of Lipodystrophy

Summary of Doctoral Thesis

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Lipodystrophy syndromes, characterized by the partial or total loss of body fat, are associated with insulin resistance, diabetes, and lipid metabolism abnormalities, including fatty liver.

Adipose tissue is an important organ, not only for energy storage, but also for metabolic regulation, and leptin, the most important adipokine secreted by adipocytes, is involved in the regulation of food intake and whole-body energy expenditure. Thus, a lack of adipose tissue is associated with low levels of circulating leptin, which result in disordered glucose and lipid metabolism.

Because the metabolic disorders that accompany lipodystrophy are ameliorated by leptin administration, leptin replacement has been approved as a treatment for lipodystrophy. Animal experiments using transgenic lipodystrophic mice have also demonstrated that leptin deficiency causes insulin resistance and that leptin replacement substantially improves insulin sensitivity.

Human recombinant leptin (metreleptin) has been reported to be effective as a leptin replacement therapy for the metabolic complications associated with lipodystrophy, and is the drug approved in the United States and Japan. Metreleptin significantly ameliorates glucose and lipid metabolic disorders in lipodystrophic patients and thereby alleviates the symptoms of lipodystrophy.

House musk shrew (*Suncus murinus*), also called suncus, is an insectivore of

the Soricidae family that has become established as a laboratory animal in Japan, and has been widely used for physiologic and morphologic experiments. One of its features is its remarkable deficiency of body fat, especially visceral fat. However, despite this, these shrews demonstrate similar blood glucose levels to humans and rodents, and unremarkable glucose metabolism. Therefore, we were interested in the reason why suncus do not show insulin resistance or other features of lipodystrophy. We hypothesized that the amount of circulating leptin and the function of this hormone might differ from that in humans and other animals.

The objectives of this study were to determine the structure of suncus leptin and its physiologic role, especially with regard to its regulation of insulin sensitivity and glucose metabolism. To this end, we cloned suncus *Lep* cDNA to determine both its and the encoded polypeptide sequence, and subsequently evaluated the tissue distribution of *Lep* mRNA in suncus.

1. Cloning of suncus *Lep* cDNA and its tissue distribution

Cloning of suncus *Lep* cDNA was carried out by combining 3' rapid amplification of cDNA ends with conventional RT-PCR of the partial 5'-untranslated region. By assembling the obtained sequences and determining a consensus sequence, the nucleotide sequence of the entire coding region of suncus *Lep* cDNA was determined.

The cloned suncus *Lep* cDNA is 3,026 bp long and contains a 513 bp putative

open reading frame that encodes a 170 amino acid (aa) polypeptide. The calculated molecular weight (MW) of the leptin precursor is 18.9 kDa. The putative suncus leptin precursor possesses a predicted signal peptide comprising 21 hydrophobic residues at its amino-terminal, suggesting that the mature leptin polypeptide is 149 aa long, with a calculated MW of 16.4 kDa.

Because suncus have less body fat than other mammals, the tissue distribution of *Lep* gene expression was evaluated to establish whether leptin might be expressed in tissues other than adipose tissue. In fact, when its tissue distribution was confirmed by RT-PCR, *Lep* gene expression was detectable only in white adipose tissue (WAT; subcutaneous and epididymal) and brown adipose tissue (BAT), which is similar to its distribution in other mammals

2. Sequence analyses and phylogenetic analysis

Next, the peptide sequence of the suncus leptin precursor was compared with its counterparts in representative mammalian species, including rat, mouse, human, horse, cow, pig, cat, and dog. The leptin sequence of the common shrew (*Sorex araneus*), also known as the Eurasian shrew, which belongs to the same family as suncus, was also compared.

The leptin precursor was found to be highly conserved among these species, with suncus leptin being homologous to the rat (77%), mouse (77%), human (75%),

horse (82%), cow (80%), pig (80%), cat (78%), dog (76%), and common shrew (Sorex) (81%) precursors. These results suggest that leptin is highly conserved in a range of mammals.

The sequence alignment revealed an insertion of 3 aa in suncus leptin that is not present in the polypeptides from the other mammals. This corresponded to a 9-base pair insertion in the *Lep* sequence of suncus, and therefore no frameshift mutation. This insertion is thought to be attributable to a 9-base microindel within the suncus *Lep* gene.

A phylogenetic tree was also constructed using mammalian leptin amino acid sequences, which included selected mammalian species, especially Placentalia. The reference leptin sequences comprised human, chimpanzee, and macaque monkey sequences to represent primates, rat and mouse sequences to represent rodents, the horse sequence to represent odd-toed ungulates, cow and pig sequences to represent even-toed ungulates, cat and dog sequences to represent carnivores, and house musk shrew and common shrew sequences to represent insectivores. The results of the phylogenetic tree analysis were very similar to those previously published.

3. 3D structure analysis

A 3D model of the suncus leptin structure was created using the established structure of the human obesity protein (leptin; PDB code: 1AX8), and this exhibited a typical four α-helix structure. These results indicate that our cloned *Lep* cDNA encodes

a suncus leptin precursor protein with a structure similar to leptin proteins in other mammals. Because the VPQ aa sequence is inserted into the CD loop, which connects helices C and D, it creates a protrusion; however, this region of the molecule is not predicted to affect binding to the leptin receptor.

Therefore, we speculated that suncus leptin has the same physiologic activity as leptin from other mammals, but for a more definitive answer, binding experiments using a recombinant protein and the leptin receptor are necessary.

In this study, we have explored the usefulness of suncus as an animal model of human lipodystrophy. To establish why suncus, which has less body fat than other mammals, does not show insulin resistance under normal conditions, we determined and analyzed the structure of suncus leptin, and identified differences from human leptin.

In the present study, the following results were obtained. cDNA cloning confirmed that suncus produces a leptin polypeptide that is highly homologous to that of other mammals. We speculate that the three-amino-acid-insertion (VPQ), which is not found in other mammalian leptins, is the result of a slippage-like event creating a microindel. The predicted 3D structure is similar to that of human leptin, but the VPQ region protrudes slightly outwards. The expression of the *Lep* gene is restricted to WAT and BAT, similar to other mammals. Detailed analysis of the function of suncus leptin using recombinant proteins is required to reveal the physiologic role of suncus leptin and

the reasons why suncus do not show insulin resistance.

In addition, in the field of veterinary medicine, there have been few reports of lipodystrophy in domestic animals. However, it is possible that in the future lipodystrophy may be identified in companion animals that are presently diagnosed with diabetes or other metabolic abnormalities. We predict that suncus will play an important role as a model for disease in dogs and cats, the commonest species seen in veterinary companion animal practice.