An animal model manifesting neurodegeneration and obesity

Etsuo Susaki¹² and Keiichi I. Nakayama¹²

¹ Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan
² CREST, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

Key words: ubiquitin, mouse model, neurodegeneration, obesity

Received: 06/27/10; accepted: 07/07/10; published on line: 07/08/10

Abstract: Although the existence of a link between neurodegenerative diseases and obesity has been suggested, a causal relation between neural degeneration and obesity has remained to be demonstrated experimentally. We recently showed that neurodegeneration in the hypothalamic satiety center results in obesity in mice transgenic for E4B (also known as UFD2a), a mammalian ubiquitin elongation factor (E4). Increased expression of E4B in neurons of the transgenic mice results in the formation of ubiquitin-positive aggregates similar to those apparent in many human neurodegenerative diseases as well as in degeneration of hypothalamic neurons responsible for the regulation of food intake and energy expenditure. We thus propose that neurodegeneration is a possible cause of human obesity and related metabolic diseases, which have become a serious public health problem worldwide. Our animal model is thus a powerful tool for studies of the relation between neurodegeneration and obesity.

Aging of the human population is a key concern worldwide because of the associated social and medical problems. Important diseases related to aging include neurodegenerative conditions, such as Alzheimer’s disease, most of which are characterized by the formation of intracellular protein aggregates in neurons and neuronal loss. Individuals with such diseases exhibit various neural abnormalities including motor, cognitive, and behavioral dysfunction. Another disease that has traditionally been associated with aging is obesity, although this condition, together with its accompanying metabolic abnormalities, has recently also begun to affect younger individuals as a result of changes in diet and lifestyle and has become a serious public health problem worldwide. A link between these two types of disease has been postulated, on the basis of their association with aging. Indeed, the possible relation between neurodegeneration and obesity in animal models or humans has been studied now for several decades. However, most such studies have focused on the possibility that obesity and related metabolic disorders exacerbate neurodegeneration and thereby promote cognitive decline and increase vulnerability to brain injury [1]. Few studies have addressed the possibility that neurodegeneration in the brain may cause obesity, as is suggested by the identification of hereditary neurodegenerative disorders associated with obesity such as Prader-Willi syndrome [2].

E4 as a new player in the ubiquitin-proteasome system

A key focus of our research group has been the functions and underlying mechanisms of the ubiquitin-proteasome system (UPS). The UPS plays an important role in the elimination of short-lived regulatory proteins [3], including those that contribute to such processes as the cell cycle, cellular signaling in response to environmental stress or extracellular ligands, morphogenesis, secretion, DNA repair, and organelle biogenesis [3-5]. The UPS pathway includes two key steps: covalent attachment of multiple ubiquitin molecules to the protein substrate and degradation of the ubiquitylated protein by the 26S proteasome
The roles of E4B in vivo have remained largely unknown, however. E4B is expressed predominantly in neural tissues of adult mice [10], suggesting that it performs a neural-specific function. We found that E4B targets the pathological form of ataxin-3—in which abnormal expansion of a polyglutamine tract is responsible for spinocerebellar ataxia type 3 (SCA3) in humans—for ubiquitylation and degradation in mammalian cells as well as in a Drosophila melanogaster model of SCA3 [14]. Furthermore, we isolated FEZ1 (fasciculation and elongation protein zeta 1), a protein implicated in neurite extension, as a binding partner of E4B [15]. FEZ1 is a mammalian homolog of Caenorhabditis elegans UNC-76, which is required for axonal bundling and elongation in the nematode [16], suggesting that a FEZ1-E4B system also participates in axonal outgrowth and fasciculation in mammals. Other groups also reported that UFD2a is implicated in the process of Wallerian degeneration of neurons [17,18]. Moreover, we showed that E4B+/− mice manifest axonal dystrophy in the nucleus gracilis as well as degeneration of Purkinje cells associated with endoplasmic reticulum stress, and that these animals develop a neurological disorder [13]. Mice nullizygous for E4B died in utero as a result of developmental defects in the heart, suggesting an additional role for E4B in developmental processes in this organ. In spite of these various observations, however, the precise physiological functions of this enzyme remained elusive.

Neurodegeneration and obesity in mice transgenic for E4B

During further studies to explore the roles of E4B, we discovered that overexpression of E4B in a neural cell line resulted in the formation of protein aggregates that were recognized by antibodies to ubiquitin as well as by those to p62, a marker of ubiquitin-associated aggregates. This phenomenon was also reproduced in E4B transgenic (Tg) mouse lines in which expression of the E4B transgene is controlled by the promoter of the gene for the mammalian prion protein [19] (Figure 1A). This aggregate formation is apparently dependent on the ubiquitylation activity of the enzyme, given that few such aggregates were detected in cells expressing E4B(ΔU), a truncated form of E4B that lacks the catalytic U-box domain (Figure 1A). In addition, an important feature of the aggregates is that they resemble ubiquitin- and p62-positive aggregates observed in many human neurodegenerative diseases or in mice with neurodegeneration resulting from defects in autophagy, another pathway for the clearance of cellular components [20,21].

The aggregate formation in E4B Tg mice was apparent specifically in certain hypothalamic nuclei. Among these nuclei, the aggregate-associated neurodegeneration was most obvious in the paraventricular nucleus (PVN). PVN neurons are activated by signaling downstream of food intake [22-25], and they function as a satiety center. Indeed, lesions in the PVN result in the development of hyperphagic obesity in rat [26]. Furthermore, neurodegeneration-associated gliosis was observed in the region adjacent to the PVN in the hypothalamus of E4B Tg mice (Figure 1B), indicating that ectopic expression of E4B results in the formation of ubiquitin-positive aggregates and associated pathological features characteristic of neurodegenerative diseases [27].

Surprisingly, the E4B Tg mice were unequivocally obese (Figure 1C) and manifested increased lipid accumulation in tissues such as adipose tissue and the liver [27]. We investigated whether this obese phenotype was attributable to functional impairment of the hypothalamic satiety center. The animals exhibited increased food intake and decreased energy expenditure as well as several abnormal responses of the center to satiety input, indicating that malfunction of the hypothalamic satiety
Our genetic mouse model has also provided the first experimental demonstration that neurodegeneration can indeed result in obesity, suggesting that some cases of human obesity might be attributable to hypothalamic neurodegeneration in aged individuals without any other neural disorders including cognitive and behavioral dysfunction. Aberrant activity of E4B might be a possible cause of obesity and associated metabolic disorders in humans, a notion that is consistent with the localization of obesity-related genetic markers in the vicinity of the E4B gene locus [30,31]. Further analysis of E4B function, particularly through identification of its substrates, should provide greater insight into the pathological properties of the molecule. More generally, nonspecific neurodegeneration associated with aging might result in a tendency to become obese. Together, our findings with E4B Tg mice open a new field of research linking obesity and aging processes as represented by degeneration of neural tissue.

**CONFLICT OF INTERESTS STATEMENT**

The authors of this manuscript have no conflict of interests to declare.
REFERENCES

1. Bruce-Keller AJ, Keller JN, Morrison CD. Obesity and vulnerability of the CNS. Biochim Biophys Acta. 2009; 1792:395-400.
2. Ristow M. Neurodegenerative disorders associated with diabetes mellitus. J Mol Med. 2004; 82:510-529.
3. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem. 1998; 67:425-479.
4. Weissman AM. Themes and variations on ubiquitylation. Nat Rev Mol Cell Biol. 2001; 2:169-178.
5. Nakayama Ki, Nakayama K. Ubiquitin ligases: cell-cycle control and cancer. Nat Rev Cancer. 2006; 6:369-381.
6. Scheffner M, Nuber U, Huibregtse JM. Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. Nature. 1995; 373:81-83.
7. Johnson ES, Ma PC, Ota IM, Varshavsky A. A proteolytic pathway that recognizes ubiquitin as a degradation signal. J Biol Chem. 1995; 270:17442-17456.
8. Koegl M, Hoppe T, Schlenker S, Ulrich HD, Mayer TU, Jentsch S. A novel ubiquitination factor, E4, is involved in multiquitin chain assembly. Cell. 1999; 96:635-644.
9. Hatakeyama S, Yada M, Matsumoto M, Ishida N, Nakayama KI. U box proteins as a new family of ubiquitin-protein ligases. J Biol Chem. 2001; 276:31111-31320.
10. Kaneko C, Hatakeyama S, Matsumoto M, Yada M, Nakayama K, Nakayama KI. Characterization of the mouse gene for the U-box-type ubiquitin ligase UFD2a. Biochem Biophys Res Commun. 2003; 300:297-304.
11. Cruciat CM, Heil K, Folsch H, Neupert W, Stuart RA. Bcs1p, an AAA-family member, is a chaperone for the assembly of the cytochrome bc1(3) complex. EMBO J. 1999; 18:5226-5233.
12. Golbik R, Lupas AN, Korette KK, Baumeister W, Peters J. The Janus face of the archaean Cdc48/p97 homologue VAT: protein folding versus unfolding. Biol Chem. 1999; 380:1049-1062.
13. Kaneko-Oshikawa C, Nakagawa T, Yamada M, Yoshikawa H, Matsumoto M, Yada M, Hatakeyama S, Nakayama K, Nakayama KI. Mammalian E4 is required for cardiac development and maintenance of the nervous system. Mol Cell Biol. 2005; 25:10953-10964.
14. Matsumoto M, Yada M, Hatakeyama S, Ishimoto H, Tanimura T, Tsuji S, Kakizuoka A, Kitagawa M, Nakayama KI. Molecular clearance of ataxin-3 is regulated by a mammalian E4. EMBO J. 2004; 23:659-669.
15. Okumura F, Hatakeyama S, Matsumoto M, Kamura T, Nakayama KI. Functional regulation of FEZ1 by the U-box-type ubiquitin ligase E4B contributes to neuritogenesis. J Biol Chem. 2004; 279:53533-53543.
16. Bloom L, Horvitz HR. The Caenorhabditis elegans gene unc-76 and its human homologs define a new gene family involved in axonal outgrowth and fasciculation. Proc Natl Acad Sci U S A. 1997; 94:3414-3419.
17. Conforti L, Tarlton A, Mack TG, Mi W, Buckmaster EA, Wagner D, Perry VH, Coleman MP. A Ufd2/D4Cole1e chimeric protein and overexpression of Rbp7 in the slow Wallerian degeneration (WldS) mouse. Proc Natl Acad Sci U S A. 2000; 97:11377-11382.
18. Mack TG, et al. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmmt chimeric gene. Nat Neurosci. 2001; 4:1199-1206.
19. Borchelt DR, Davis J, Fischer M, Lee MK, Slunt HH, Ratovitsky T, Regard J, Copeland NG, Jenkins NA, Sisodia SS, Price DL. A vector for expressing foreign genes in the brains and hearts of transgenic mice. Genet Anal. 1996; 13:159-163.
20. Hara T, Nakamura K, Matsu M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature. 2006; 441:885-889.
21. Komatsu M, et al. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell. 2007; 131:1149-1163.
22. Kim MS, et al. Hypothalamic localization of the feeding effect of agouti-related peptide and alpha-melanocyte-stimulating hormone. Diabetes. 2000; 49:177-182.
23. Kublaoui BM, Holder JL, Jr., Gemelli T, Zinn AR. Sim1 haploinsufficiency impairs melanocortin-mediated anorexia and activation of paraventricular nucleus neurons. Mol Endocrinol. 2006; 20:2483-2492.
24. Sarkar S, Legradi G, Lechan RM. Intracerebroventricular administration of alpha-melanocyte stimulating hormone increases phosphorylation of CREB in TRH- and CRH-producing neurons of the hypothalamic paraventricular nucleus. Brain Res. 2002; 945:50-59.
25. Wirth MM, Olszewski PK, Yu C, Levine AS, Giraudo SQ. Paraventricular hypothalamic alpha-melanocyte-stimulating hormone and MTII reduce feeding without causing aversive effects. Peptides. 2001; 22:129-134.
26. Leibowitz SF, Hammer NJ, Chang K. Hypothalamic paraventricular nucleus lesions produce overeating and obesity in the rat. Physiol Behav. 1981; 27:1031-1040.
27. Susaki E, Kaneko-Oshikawa C, Miyata K, Tabata M, Yamada T, Oike Y, Katagiri H, Nakayama KI. Increased E4 activity in mice leads to ubiquitin-containing aggregates and degeneration of hypothalamic neurons resulting in obesity. J Biol Chem. 2010; 285:15538-15547.
28. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998; 395:763-770.
29. Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. Neuron. 1999; 22:221-232.
30. Deng HW, Deng H, Liu YJ, Liu YZ, Xu FH, Shen H, Conway T, Li JL, Huang QY, Davies KM, Recker RR. A genomewide linkage scan for quantitative-trait loci for obesity phenotypes. Am J Hum Genet. 2002; 70:1138-1151.
31. Stone S, et al. A major predisposition locus for severe obesity, at 4p15-p14. Am J Hum Genet. 2002; 70:1459-1468.