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AT-A-GLANCE ARTICLE

MicroRNAs in the hypothalamic control of energy homeostasis

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Obesity is a medical condition characterized by excess of fat content in the body. The majority of obese patients additionally develop cardiovascular disease and type 2 diabetes mellitus collectively referred to as the metabolic syndrome. Such condition reduces life expectancy and may ultimately result in cancer development. It affects more than half a billion adults worldwide and is a constantly growing epidemic, especially in developed and developing countries. This can be largely attributed to the lack of genetic, epigenetic, and social resistance to the unprecedented availability of cheap carbohydrate and fat rich food. Metabolic challenges such as overeating or fasting transiently or chronically disrupt energy homeostasis which is maintained by a tightly regulated communication between peripheral organs and the central nervous system. In the former, the key regulatory functions are attributed to the pancreas, the main endocrine organ in the glucose metabolism, fat-storing cells called adipocytes, and the liver, the metabolic factory of our body. Integration of all signals from the peripheral organs and control of feeding behavior and weight are governed by the brain, where the major regions involved in metabolism are located within the hypothalamus. It contains a number of nuclei crucial for hunger and satiety responses. The homeostatic balance is achieved at several levels: (i) hormonal, by soluble factors signaling throughout the body; (ii) neuronal, by a complex network of neural cells; (iii) transcriptional, regulation of gene message expression in the cell nucleus; and (iv) at the post-transcriptional level, where mRNA and protein levels are regulated outside of the cell nucleus. MicroRNAs represent a well-characterized class of small non-coding RNAs which can degrade gene messages or abrogate protein translation. In this work, we briefly reviewed the role of microRNAs in the energy homeostasis control by the hypothalamus.

The hypothalamic regions regulating energy homeostasis

The worldwide increasing rate of obesity and associated metabolic disorders has led to a growing demand of exploration and validation of novel and already established causative factors and their interactions, which ultimately could be manipulated to treat the pathologies of disrupted energy balance. The interplay between the central nervous system and energy balance has been investigated extensively for many decades. The hypothalamus is the most studied energy balance-associated region of the brain due to its direct involvement in both sensing the metabolic states and control of feeding and energy expenditure. The anatomical location and diverse inter-connected neuronal populations enable this small region of the brain to act as a channel between the nervous and endocrine systems, regulating the energy balance of the whole body. In particular, the arcuate nucleus of the hypothalamus (ARC) is one of the core units easily accessible for the peripheral hormonal and nutrient signals including leptin, ghrelin, insulin, glucose, fatty acids, and amino acids. The two mostly studied types of neurons within this nucleus which functionally antagonize each other are (i) the fast-acting food intake-stimulating (or orexigenic) neuropeptide Y (NPY)- and

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agouti-related protein (AgRP)-expressing neurons, and (ii) the slow-acting, food intake-inhibiting (or anorexigenic) proopiomelanocortin (POMC) and its derivative α-melanocyte-stimulating hormone (α-MSH)-expressing neurons (Garfield et al. 2015; Motojima et al. 2016; Ong et al. 2017; Shah et al. 2014). Recently, as a fast anorexigenic, counter-acting to AgRP neurons, the new subtype of glutamatergic neurons expressing oxytocin receptor and vesicular glutamate transporter 2 has been identified within ARC (Fenselau et al. 2017). These three neuronal populations are the first-order neurons to sense peripheral queues such as hormones or metabolites and engage the second-order neurons such as oxytocin neurons within the paraventricular nucleus (PVN) within and outside of the hypothalamus by releasing neurotransmitters and neuropeptides.

Notably, together with NPY, AgRP, and α-MSH, oxytocin is one of the major regulators of energy homeostasis. Indeed, in addition to very well-studied melanocortin 4 receptor (MC4R) anorexigenic neurons, oxytocin neurons were shown to be very important in appetite suppression and energy expenditure stimulation (Hume et al. 2017; Kublaoui et al. 2008; Wu et al. 2012; Zhang and Cai 2011). Moreover, expression of oxytocin MC4R overlaps in PVN neurons. In addition, these PVN anorexigenic neurons receive projections from ARC POMC, glutamatergic, and AgRP neurons and can rapidly decrease food intake and increase energy expenditure upon their activation (Atasoy et al. 2012; Caquineau et al. 2006; Fenselau et al. 2017). The PVN oxytocin neurons→ ARC POMC neuron projections have been also identified suggesting yet another intriguing hypothalamic feedback regulation (Maejima et al. 2014). Such a complex neuronal circuit enables a rapid integrated response to daily changes in body metabolic state such as hunger and satiety (Fig. 1).

Dicer in CaMKII, POMC, and other neurons

Dicer is a ubiquitously expressed protein especially crucial for completion of the developmental program of the organism. Together with Drosha, they are the key endoribonucleases in microRNA biogenesis. With aging, Dicer expression decreases in the white adipose tissue which can be prevented by calorie restriction, and in the brain (Chmielarz et al. 2017; Domanskyi and Vinnikov 2017; Mori et al. 2014). Moreover, Dicer is the only component of the microRNA biogenesis machinery that reacts to decreased nutrient availability in the hypothalamus during fasting. Additionally, in diet-induced or genetic models (*ob*/*ob*) of obesity, Dicer expression decreases. Above observations indicate that the intense processing of information inside the cells engaged in generating metabolic orders for the whole organism depends, at least, partially on non-coding, Dicer-dependent RNAs (Schneeberger et al. 2012). Our previous studies demonstrated that microRNAs are crucial for plastic changes in neurons during formation of memory engram (Konopka et al. 2010). In order to prove that microRNAs serve a critical function in metabolically active neurons, the *Dicer1* gene was removed from forebrain neurons, including those within the hypothalamus by application of inducible, cre/lox system (Fiorenza et al. 2016; Mang et al. 2015; Vinnikov et al. 2014). The major advantage of Dicer^{CaMKCreERT2} (further also referred as DicerCKO) approach is that mutation is induced specifically in adult animals. This leaves the developmental processes in cells, which are largely dependent on microRNAs, intact. We have discovered that the removal of microRNAs exclusively from ARC neurons leads to severe hyperphagia and obesity on the regular chow diet and that this phenotype critically depends on chronic over-activation of the mTOR pathway (Vinnikov et al. 2014). This phenotype was further confirmed in two independent laboratories (Fiorenza et al. 2016; Mang et al. 2015). In the laboratory of Angel Barco, it was shown that upon removal of microRNAs, neurons show increased responsiveness. The authors postulate that the obesity phenotype is induced by hyper-activity of hypothalamic neurons that additionally become insensitive to applied ghrelin in Dicer-depleted animals (Fiorenza et al. 2016). Moreover, delineation of specific microRNA, miR-103, capable to attenuate both mTOR over-activation and hyperphagic obesity in DicerCKO animals allowed us to make the first step towards identification of the mechanism behind the obesity phenotype in these mice (Vinnikov et al. 2014).

Deletion of the *Dicer1* gene in POMC neurons leads to the development of obesity (Greenman et al. 2013; Schneeberger et al. 2012). Those studies were conducted with a regular, not inducible cre/lox system and hence, Dicer deletion occured early in development, severely affecting proper differentiation of POMC cells leading to their loss. The interpretation of the results is further hampered by pituitary defects and changed stress responses. Altogether, above experiments point to the crucial role of microRNAs in the development of neurodegenerative obesity. Of note, the POMC neurons in our DicerCKO model appear to be preserved, thus excluding the possibility that the obesity phenotype results directly from the loss of this type of cells.

Insulin and leptin—two hormones reporting about the energy status—bind respective receptors on the cell membrane and activate intracellular pathways which induce specific programs of gene expression such as POMC or AgRP genes. Inside the cell, specific elements of these pathways partially overlap to functionally coordinate neuronal responses. MicroRNAs as translational regulators serve as perfect candidates to control the levels of particular components of intracellular signaling pathways, i.e., JAK-STAT or PI3K-AKT-mTOR. Additionally, leptin sets perinatally the adiposity level and protects against obesity later during adulthood (Pico et al. 2011). This signaling molecule regulates activity of a variety

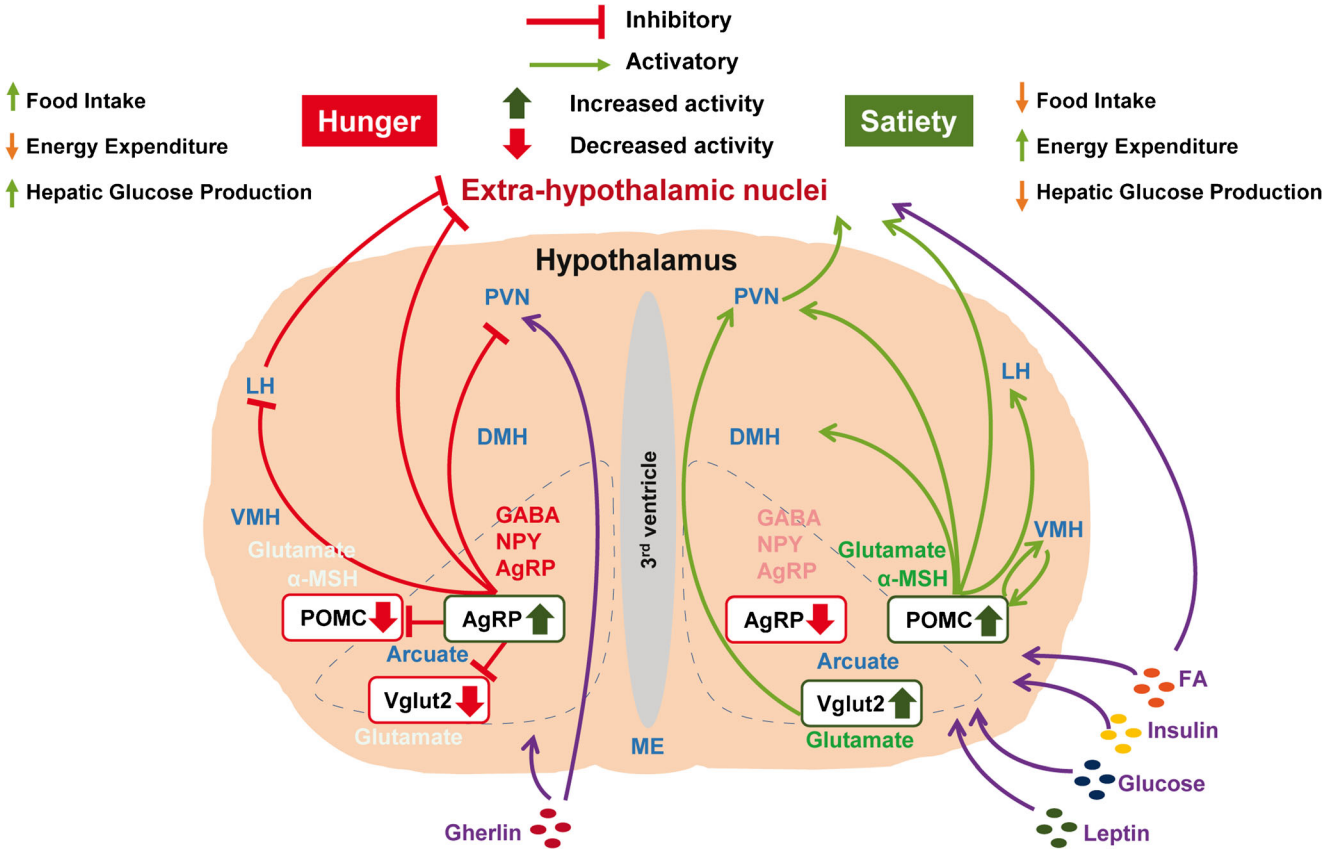

Fig. 1 Hypothalamic control of hunger and satiety. A schematic representation of the nuclei of the hypothalamus involved in energy homeostasis control. In hunger state (left), upon sensing the periphery signals, AgRP neurons release GABA, NPY, and AgRP which inhibit POMC neurons; Vglut2 neurons; and neurons within VMH, DMH, LH, and PVN, as well as satiety neurons in extra-hypothalamic nuclei to enhance feeding. In satiety state (right), the AgRP neurons activity is suppressed while both POMC and Vglut2 neurons get activated by sensing signals both from the periphery and the brain and release α-MSH and glutamate to activate hypothalamic and extra-hypothalamic neurons and suppress feeding and stimulate energy expenditure. α-MSH, α-melanocyte-stimulating hormone; AgRP, agouti-related protein; DMH, dorso-medial nucleus of the hypothalamus; FA, fatty acids; GABA, γ-aminobutyric acid; LH, lateral hypothalamus; ME, medial eminence; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, periventricular nucleus of the hypothalamus; Vglut2, vesicular glutamate transporter 2; VMH, ventromedial nucleus of the hypothalamus

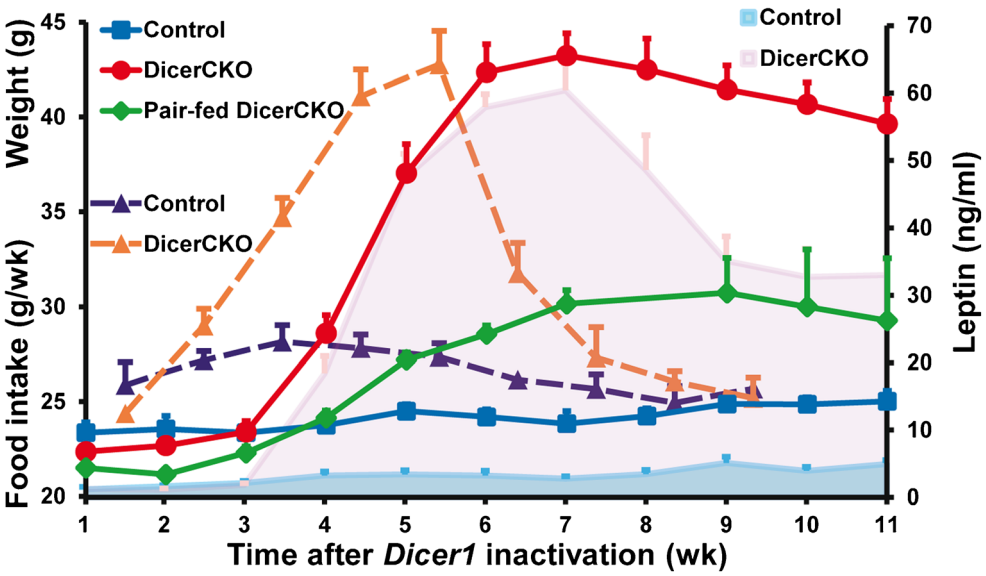

Fig. 2 Time course of weight, food intake and plasma leptin levels in DicerCKO mice. The figure summarizes the data published in (Vinnikov et al. 2014). *Dicer1* deletion in $Dicer^{CaMKCreERT2}$ mice was induced by tamoxifen injections. Additionally, weight gain curve of pair-fed DicerCKO animals is presented (green) when the animals were fed daily with the amount of food matched with the consumption of the control group. Note the critical (but not exclusive—see the pair-fed group) role of early food intake increase in the observed phenotype and its effective normalization upon plasma leptin concentration increase

of neurons including POMC neurons. It was demonstrated that leptin is necessary for adjusting the expression of the set of microRNAs targeting 3′-UTR of POMC mRNA, e.g., miR-488. This microRNA is upregulated in both *ob*/*ob* and *db*/*db* genetic mouse models of obesity with disrupted leptin signaling. Application of leptin to *ob*/*ob* mice downregulated the expression level of miR-488 (Derghal et al. 2015). It is very likely that leptin brings the balance to the energy regulatory system in Dicer^{CaMKCreERT2} mice (Fig. 2).

MicroRNAs and intracellular pathways regulating the energy homeostasis

We have found that in DicerCKO mice following tamoxifen induced *Dicer1* deletion, there is a gradual increase in the mTOR kinase activity as measured by phosphorylated form of S6 ribosomal protein levels (Vinnikov et al. 2014). In silico analysis of hypothalamus-expressed microRNAs revealed a set of microRNAs potentially targeting elements of the PI3K-Akt-mTOR pathway. In vitro experiments confirmed that selective knock-down of those microRNAs results in over-activation of mTOR activity for some of them, e.g., miR-103. Delivery of chemically stabilized oligonucleotides mimicking the set of microRNAs or miR-103 mimic individually into the brain, significantly reduced phosphorylation of S6 protein, food intake, and weight gain in the mutant animals (Vinnikov et al. 2016; Vinnikov et al. 2014). Interestingly, over-activation of the mTOR pathway upon aging or after inactivation of TSC1 (negative regulator of mTOR) in POMC neurons stimulates ATP-dependent K_{ATP}-channels causing their hyperpolarization and inactivation, which leads to hyperphagia and obesity (Yang et al. 2012). Another example of microRNAs upregulated in the hypothalamus of *ob*/*ob* mice is miR-200a. Leptin treatment of *ob*/*ob* mice reduces leptin and insulin signaling impairment by increased expression of leptin receptor and insulin receptor substrate 2, respectively (Crepin et al. 2014). Finally, the miR-200a silencing in the brain reduced body weight gain.

Summary

Despite a vast number of studies, we are still far from understanding the interplay of various factors in energy homeostasis control by the hypothalamus. In this work, we tried to highlight the role of hypothalamic microRNAs in the metabolic control. Undoubtedly, the major role in the prevention of adiposity is sensible nutrition and healthy lifestyle. However, most of the patients find it difficult to overcome their habits, while a plethora of therapeutic approaches available to date, excluding sleeve gastrectomy and similar surgical interventions, are ineffective. Further studies might open new avenues for pharmacological treatment of metabolic syndrome conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with mouse or human participants performed by any of the authors.

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