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REVIEW

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Nesfatin-1: a novel satiety factor and its molecular cloning applications

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ABSTRACT

Given the increasing health challenges posed by conditions like obesity and diabetes, a comprehensive understanding of appetite regulation and metabolic processes is crucial. Nesfatin-1 is a vital satiety factor governing hunger, satiety, and energy balance. Its role in glucose homeostasis, insulin sensitivity is well established, and cloning of this gene has enabled a wide range of applications, ranging from fundamental research elucidating appetite regulation mechanisms to translational studies aiming at innovative therapies for obesity and metabolic disorders. However, the cloning of the gene has led to a number of challenges, such as post-translational modifications and diverse splicing variants. This review aims to identify and characterize these variants to understand their specific functions and regulatory mechanisms and address challenges in nesfatin-1 cloning strategies, focusing on current splicing methods and proposing advanced techniques like single-cell RNA sequencing and CRISPR/Cas9-mediated genome editing. Collaborative efforts and technological progress, rooted in a deep nesfatin-1 understanding, are key to enhancing the quality of life for those affected, signaling a significant step towards a healthier future.

KEYWORDS

Appetite regulation; CRISPR-Cas; Biomarkers; Drug development; Sequence analysis; Cloning

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Introduction

Food intake and energy balance regulation is a complex and highly regulated process essential for an organism's survival and overall health. It involves the integration of peripheral neuronal, metabolic, and endocrine signals, with the gut and brain serving as two pivotal tissues expressing many appetite-regulating peptides. These peptides, collectively known as satiety factors, play a fundamental role in the intricate network that governs hunger and satiety [1]. Among these factors, nesfatin-1, a peptide derived from the NUCB2 gene, has emerged as a significant player in appetite control. Nesfatin-1's unique ability to traverse the blood-brain barrier and influence multiple physiological processes positions it as a potent regulator of food intake and energy balance [2,3]. Understanding the mechanisms underlying satiety factors is crucial, as they control appetite and energy homeostasis. Their implications address the prevalent metabolic disorders such as obesity and diabetes.

This review aims to provide an in-depth analysis of nesfatin-1, a crucial satiety factor involved in appetite regulation and metabolic processes. The primary objective is to understand the molecular structure of nesfatin-1 and its role in appetite regulation. The focus is on addressing challenges in nesfatin-1 cloning methods, such as post-translational modifications and diverse splicing variants, and proposing advanced techniques like single-cell RNA sequencing and CRISPR/Cas9-mediated genome editing.

Satiety Factors: Defining the Significance

Satiety factors are the messengers dispatched to communicate with the brain, signaling hunger and fullness. These endocrine factors, often classified as either appetite-stimulating (orexigenic) or appetite-inhibiting (anorexigenic) peptides, are key components of the intricate system (central nervous system and the peripheral nervous systems) that govern food intake and energy expenditure [4]. They are present not only in the central nervous system (CNS) but also in the peripheral nervous systems, creating a web of communication that ensures the body's nutritional needs are met [5].

The hypothalamus is the primary site where satiety factors exert their influence, a critical region within the brain responsible for regulating feeding behavior [1]. As it is located near the median eminence, one of the circumventricular organs, this hypothalamus region receives hormonal cues from the periphery via systemic circulation [6]. Within the hypothalamus, specific neuronal populations known as "first-order neurons" are the primary processors of orexigenic and anorexigenic signals, regulating hunger and satiety [7].

Among the first-order neurons, anorexigenic neurons produce proopiomelanocortin (POMC) and cocaine and amphetamine-related transcripts (CART), inhibiting appetite [8]. On the other hand, orexigenic neurons express neuropeptide Y and agouti-related peptide (NPY/AgRP), promoting appetite stimulation [9]. These neuronal populations are not limited to the arcuate nucleus (ARC) of the hypothalamus but are also found in regions such as the paraventricular nucleus (PVN), ventromedial nucleus (VMN), and lateral hypothalamus (LH) [10]. This intricate appetite regulation system involves a complex interplay of behavioral, hormonal, and dietary inputs, connecting the periphery with higher cerebral regions to maintain the body's energy balance [10].

Nesfatin-1 as a Potent Satiety Factor

OhI and colleagues elucidated the molecular mechanisms of appetite regulation that led to the discovery of nesfatin-1 [1]. This peptide, derived from NUCB2, uniquely regulates food

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RESEAPRO JOURNALS

intake and energy balance by traversing the blood-brain barrier without saturation, making it a potent regulator of food intake [3]. Beyond the hypothalamus, nesfatin-1 influences various physiological processes, including energy expenditure and metabolic homeostasis. This makes nesfatin-1 a key player in the complex interplay between peripheral signals and central appetite control.

Nesfatin-1 on glucose homeostasis

Nesfatin-1, encoded by the NUCB2 gene, plays a role in the regulation of nutrient intake and exerts significant influence over glucose metabolism. The presence of NUCB2/nesfatin-1 alongside insulin in the human and rodent pancreas signifies its role in glycemic control. Nesfatin-1 in glucose metabolism has been extensively studied, revealing its multifaceted impact on blood glucose levels and insulin sensitivity [11].

Enhanced insulin secretion

Nesfatin-1's ability to augment glucose-induced insulin secretion defines its role as a glucose-dependent insulinotropic peptide. In vitro studies have demonstrated that nesfatin-1 increases the expression of pre-proinsulin mRNA, contributing to enhanced insulin production. Moreover, nesfatin-1 facilitates insulin secretion by stimulating calcium flow, primarily involving L-type channels. This mechanism highlights nesfatin-1's direct impact on insulin release from pancreatic cells [12].

Antihyperglycemic effects

Nesfatin-1's potential in glucose regulation is evident from its antihyperglycemic effects, especially in diabetic models. Intravenous administration of nesfatin-1 significantly reduces blood glucose levels in hyperglycemic db/db mice, mimicking type 2 diabetes [13]. Furthermore, continuous subcutaneous infusion of nesfatin-1 during an oral glucose tolerance test (OGTT) effectively decreases blood glucose levels. These findings emphasize nesfatin-1's ability to counter hyperglycemia, providing a solution for diabetes management [14].

Insulin sensitivity and glucagon regulation

Nesfatin-1 enhances insulin sensitivity by modulating insulin and glucagon levels [14]. Continuous subcutaneous infusion of nesfatin-1 increases circulating insulin levels and decreases glucagon levels during OGTT, suggesting an improvement in insulin sensitivity. These observations imply that nesfatin-1 enhances insulin's effectiveness, promoting efficient glucose uptake and utilization by peripheral tissues [15].

Clinical relevance

Clinical studies confirm nesfatin-1's significance in glucose homeostasis. Patients with type 2 diabetes exhibit decreased plasma nesfatin-1 levels compared to healthy individuals, emphasizing its potential as a biomarker for diabetes [16]. Similarly, pregnant women with gestational diabetes mellitus show lower serum nesfatin-1 levels, indicating its relevance in gestational diabetes [17].

Peripheral vs. central administration

Peripheral administration of nesfatin-1, particularly through subcutaneous infusion, exhibits significant effects on glucose metabolism. In contrast, central administration has minimal impact on blood glucose levels, highlighting peripheral nesfatin-1 actions in glycemic control [14].

Structure of Nesfatin-1

In recent years, the field of molecular biology has witnessed the discovery of numerous regulatory neuropeptides, each with its unique characteristics and broad spectrum of physiological activities. Among these, nesfatin-1, also known as NEFA or NUCB2-encoded satiety and fat-influencing protein, is composed of 82 amino acids derived from the precursor protein nucleobindin-2 (NUCB2) [18]. The NUCB2 gene product is a peptide with 420 amino acids (AA), composed of a 396 AA long precursor peptide and a 24 AA long signal peptide (SP) [19]. The conversion of NUCB2 into nesfatin-1 involves proteolytic cleavage by prohormone/proprotein convertases (PC) 1/3 and PC2, resulting in three distinct peptides: nesfatin-1 (AA 1-82), nesfatin-2 (AA 85-163), and nesfatin-3 (AA 166-396), as shown in Figure 1. Among these, nesfatin-1 is the only exhibit biological activity [1].

The structure of nesfatin-1 is divided into three parts:

1. N23 (N-terminal 23 amino acids)
2. M30 (middle 30 amino acids)
3. C29 (C-terminal 29 amino acids)

Among them, M30 has been identified as critical for the anorexigenic (appetite-suppressing) action of nesfatin-1. However, no obvious biological activity has been detected for nesfatin-2 or nesfatin-3 [20].

Nucleobindin is a Ca2-binding polypeptide associated with DNA and regulatory proteins. It is involved in several signaling cascades in the cellular system [21]. Nucleobindin was discovered for its ability to bind to DNA fragments in vitro, hence identified as a transcription factor. Furthermore, two nucleobindins have been identified: nucleobindin-2 (NUCB2) and nucleobindin-1 (NUCB1) [19,22]. Despite being encoded by two unique and unlinked genes, mammalian NUCB1 and NUCB2 share 62 percent of their amino acid sequence [23].

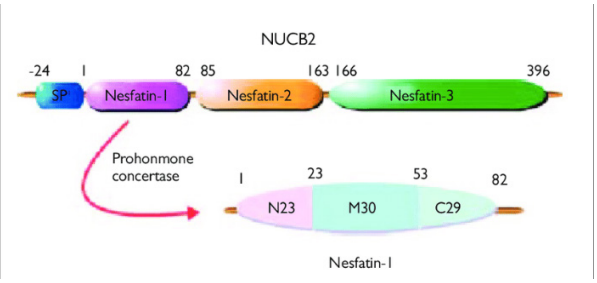

Figure 1. Nesfatin-1 is derived from the structural composition of the NUCB2 gene [24].

Distribution of nesfatin-1 in the central nervous system

Nesfatin-1 is expressed in several regions of the CNS of mammals and non-mammals, including the forebrain, hindbrain, brainstem, and spinal cord, as demonstrated in Tables 1 and 2 [25]. In mammals, Nesfatin-1 immunoreactive cells are detected in various nuclei such as ARC, PVN, and supraoptic nucleus (SON) [26-31]. These nuclei are crucial components of hypothalamic pathways that regulate food intake and energy homeostasis. In the ARC,

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Nesfatin-1-immunoreactive cells are colocalized with other important neuropeptides, including POMC, CART, NPY, oxytocin, and vasopressin [32,33]. This suggests that Nesfatin-1 plays a role in the intricate balance of appetite regulation. Additionally, in non-mammals, particularly in fish, two isoforms of NUCB2, namely NUCB2A and NUCB2B, are expressed [15]. NUCB2A predominates in fish [15]. Fish brain regions, including the nucleus lateralis tuberis (NLT) akin to the mammalian ARC, exhibit Nesfatin-1 expression, crucial for regulating food intake, growth, and energy balance [34].

Table 1. Overview of Nesfatin-1 tissue distribution in mammals.

Category	Mammalian	References
Nesfatin-1 function	Primarily regulates energy homeostasis.	[35]
Brain regions	PVN (Paraventricular nucleus) ARC (Arcuate nucleus) LH (Lateral hypothalamus) SON (Supraoptic nucleus) NTS (Nucleus tractus solitarius) EW (Edinger westphal) DMNV (Dorsal motor nucleus of the vagus) Caudal raphe nuclei	[26-31]
Associations	Associated with neuropeptide appetite regulators such as POMC, NPY, and others.	[32,33]
Peripheral tissues	Found in pancreatic betacells, adipose tissue, stomach, and gastric endocrine glands.	[15,27]
Expression changes	Expression changes observed in response to factors like starvation.	[15]
Bloodbrain barrier	Nesfatin-1 may cross the bloodbrain barrier and have central effects.	[11]

Table 2. Overview of Nesfatin-1 tissue distribution in non-mammals.

Category	Non- Mammalian (Fish)	References
Isoforms in fish	Fish express two NUCB2 isoforms: NUCB2A and NUCB2B. NUCB2A is predominant.	[15]
Fish brain regions	In fish, NLT is similar to the mammalian ARC and is involved in food intake, growth, and energy balance. Nesfatin-1 expression found in brain regions, including during starvation.	[15]
Fish tissues	Nesfatin-1 expression detected in various fish tissues, with varying levels, e.g., liver, pituitary, muscles, and gills.	[15,36]
Expression in other non-mammals	Found in frog brain and olfactory bulb during starvation. Expression in zebrafish anterior gastrointestinal tract mucosal layer cells.	[34]
Immunoreactivity	Immunoreactivity of NUCB2 confirmed in the pituitary gland of goldfish, ovarian cells, and follicular cells of zebrafish.	[15]

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Mechanism of Action

The physiological effects of nesfatin-1 are mediated through its interaction with a G-protein coupled receptor. Nesfatin-1 exerts its influence on specific neuronal populations, notably in the ARC and PVN of the hypothalamus [11]. In the ARC, pivotal for appetite control, two types of neurons, NPY and AgRP neurons, act as orexigenic signals, stimulating appetite and food intake [37,38]. Nesfatin-1 plays a crucial role in instigating an inhibitory hyperpolarization effect on these NPY/AgRP neurons [39]. Upon binding to its receptor on the neuron's surface, nesfatin-1 initiates a cascade of intracellular events leading to hyperpolarization [40]. This phenomenon involves a change in the neuron's membrane potential, making it more negative and less likely to generate an action potential. Consequently, the activity of NPY/AgRP neurons is reduced [40]. By inhibiting these orexigenic neurons, nesfatin-1 significantly diminishes appetite and food intake, underscoring its anorexigenic, or appetite-suppressing, properties. This intricate mechanism illustrates how nesfatin-1 intricately modulates neuronal activity to regulate appetite and maintain energy balance. Similarly, PVN is another crucial region in the brain involved in appetite regulation. One of the key pathways in the PVN that modulates appetite is the melanocortin signaling pathway. Melanocortins, such as alpha-melanocyte-stimulating hormone (α-MSH), are appetite-suppressing peptides [39]. They exert their effects by binding to melanocortin receptors (MC3R and MC4R) in the PVN [39]. Upon interaction with the PVN, nesfatin-1 intricately modulates the activity of these melanocortin receptors [39]. This modulation, occurring through complex interactions with the receptors and their downstream signaling pathways, amplifies nesfatin-1's appetite-suppressing effects. By influencing the melanocortin signaling within the PVN, nesfatin-1 contributes substantially to the regulation of appetite, leading to reduced food intake and ultimately influencing overall energy balance [39]. These actions ultimately lead to a reduction in food intake and body weight.

Molecular Cloning Techniques in Nesfatin-1

Cloning the nesfatin-1 gene involves a series of molecular techniques aimed at isolating, amplifying, and studying the specific DNA sequence responsible for encoding the nesfatin-1 peptide. Here is a detailed breakdown of how various cloning techniques are applied in nesfatin-1 research:

Gene isolation

The process begins with the isolation of genomic DNA containing the nesfatin-1 gene from the target organism's cells. This DNA is purified and then treated with restriction enzymes. These enzymes cut the DNA at specific recognition sites, resulting in fragments of varying sizes [41].

Polymerase chain reaction (PCR)

To specifically amplify the nesfatin-1 gene, PCR is employed. Primers designed to match the nesfatin-1 gene's sequence flank the region of interest. Through a series of temperature cycles, the DNA is denatured, primers anneal to the target sequence, and DNA polymerase extends the primers, creating multiple copies of the gene fragment [42].

Cloning vectors

The amplified nesfatin-1 gene fragment needs a suitable vector to facilitate cloning. Plasmids, circular DNA molecules often derived from bacteria, are commonly used. The nesfatin-1 fragment and the plasmid are cut with the same restriction enzyme, creating compatible sticky ends, ensuring the nesfatin-1 fragment can be integrated into the plasmid vector [43].

Ligation and transformation

The cut nesfatin-1 gene fragment is ligated into the cut plasmid vector using DNA ligase. This creates a recombinant DNA molecule where the nesfatin-1 gene is inserted into the plasmid. This recombinant plasmid is then introduced into host cells, usually bacteria like Escherichia coli, through a process called transformation [44].

Selection and screening

Bacteria that have taken up the recombinant plasmid are selected using antibiotic resistance markers present in the plasmid. These bacteria are then screened to identify colonies containing the nesfatin-1 gene. This screening can involve techniques like colony PCR, where DNA from individual colonies is amplified and checked for the presence of Nesfatin-1 DNA [41].

DNA sequencing

Once a positive clone is identified, the nesfatin-1 gene sequence is confirmed through DNA sequencing. This step ensures the accuracy of the cloned gene and provides detailed information about its nucleotide composition [41].

Expression studies

In functional studies, the cloned nesfatin-1 gene can be expressed in host cells to produce the nesfatin-1 peptide. This can involve inducing the host cells to express the gene and subsequently purifying the produced nesfatin-1 peptide for further biochemical and physiological analyses [41].

These precise molecular cloning techniques, when employed meticulously, allow researchers to isolate, clone, and study the nesfatin-1 gene and its corresponding peptide, enabling a deeper understanding of its functions in satiety regulation and related physiological processes.

Applications of Cloning Nesfatin-1

Understanding satiety mechanisms

Cloning nesfatin-1 allows for the detailed study of its genetic structure and regulation [45]. Understanding the gene's sequence helps to understand the mechanisms behind satiety, explaining how nesfatin-1 influences appetite control and energy balance.

Functional analysis of nesfatin-1

Cloned nesfatin-1 genes can be expressed in various host cells, enabling the production of the nesfatin-1 peptide in controlled environments [45]. This synthesized peptide can then be used for functional studies, elucidating its effects on neuronal circuits, hormonal interactions, and metabolic pathways [46]. These studies provide insights into how nesfatin-1 modulates hunger and satiety signals.

Drug development and therapeutics

Cloning nesfatin-1 is instrumental in drug discovery. The cloned gene can be manipulated to develop analogs or

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antagonists that influence nesfatin-1's activity. These compounds hold potential as therapeutic agents for obesity, eating disorders, and metabolic diseases, providing novel pathways for pharmaceutical interventions [47].

Genetic and epigenetic studies

Cloning nesfatin-1 facilitates genetic and epigenetic investigations. Comparative genomics using cloned nesfatin-1 sequences from different species reveals evolutionary patterns [48]. Additionally, epigenetic modifications associated with nesfatin-1, such as DNA methylation patterns, can improve understanding of gene regulation in appetite control [49].

Animal models and functional studies

Cloned nesfatin-1 genes enable the creation of transgenic animal models. Introducing or silencing the gene allows to observe behavioral and physiological changes, providing crucial insights into obesity, diabetes, and related disorders [50]. These models serve as crucial platforms for testing potential therapies.

Clinical biomarker development

Studying cloned nesfatin-1 sequences from individuals with different metabolic states can lead to the identification of genetic variations associated with obesity and related diseases [17]. These variations can serve as potential biomarkers, aiding in the early diagnosis and personalized treatment of metabolic disorders [17].

Nutritional research and dietary interventions

Cloned nesfatin-1 genes contribute to nutritional research by elucidating how dietary components influence gene expression and peptide production. Understanding these interactions can inform the development of dietary interventions that modulate nesfatin-1 levels, promoting satiety and healthy weight management.

The cloning of Nesfatin-1 enables diverse applications, ranging from fundamental research elucidating appetite regulation mechanisms to translational studies aiming at innovative therapies for obesity and metabolic disorders [51].

While the cloning of the Nesfatin-1 gene has significantly advanced our understanding of appetite regulation and metabolic processes, several gaps and challenges persist in current cloning strategies. One major hurdle lies in accounting for the Post-Translational Modifications (PTMs) like glycosylation and phosphorylation that nesfatin-1 undergoes, impacting the peptide's biological activity [52]. Incorporating these PTMs into cloning strategies is a crucial gap, as it would provide a more accurate representation of Nesfatin-1's physiological functions. Additionally, Nesfatin-1 exhibits multiple splicing variants, each potentially playing distinct roles in appetite regulation [53]. Conventional cloning methods may not capture the diversity of these variants comprehensively. There is a need for advanced techniques to identify, isolate, and characterize these variants to understand their specific functions and regulatory mechanisms [41]. Tissue-specific expression and regulation further complicate matters, with cloning methods often limited to specific tissues, failing to grasp the full scope of nesfatin-1's regulatory network. Furthermore, nesfatin-1 operates within intricate signaling pathways and interacts with various molecules and receptors, interactions frequently neglected in cloning studies, thereby providing an incomplete understanding of its downstream effects [53]. Notably, nesfatin-1's functions vary significantly across species, a factor often disregarded in cloning strategies. Human populations exhibit genetic variability, which can influence Nesfatin-1 expression and function [54]. Existing cloning strategies might not adequately capture this variability, leading to potential gaps in understanding Nesfatin-1's role in diverse human populations. Studying the genetic and ethnic variations in Nesfatin-1 sequences is vital for personalized medicine approaches [55].

Addressing these gaps necessitates the development of advanced cloning techniques, such as single-cell RNA sequencing, CRISPR/Cas9-mediated genome editing, and comprehensive multi-omics approaches [56]. Integrating multi-omics approaches will enable a comprehensive understanding of Nesfatin-1's complexities, paving the way for personalized interventions in appetite regulation and metabolic disorders, a promising frontier in the pursuit of targeted therapies [57].

Conclusions

The study of Nesfatin-1 is pivotal in understanding appetite regulation and metabolism. This intricate satiety factor connects peripheral signals to central appetite control, profoundly impacting hunger, satiety, and energy balance. Despite progress in cloning the Nesfatin-1 gene, challenges persist, including post-translational modifications, splicing variants, tissue-specific regulation, and species differences. Addressing these challenges is vital, and advanced techniques like single-cell RNA sequencing and CRISPR/Cas9 genome editing offer promise [57]. Integrating multi-omics approaches provides a holistic understanding, providing a comprehensive view of genetic, epigenetic, and proteomic aspects concurrently.

Nesfatin-1 research has diverse applications, from drug development to personalized medicine based on genetic variations. Transgenic animal models elucidate obesity and diabetes. This gene's cloning expands our knowledge and lays the foundation for targeted therapies. Collaborative efforts and technological advancements ensure a promising future, revolutionizing appetite regulation and metabolic disorders for improved quality of life.

Disclosure statement

No potential conflict of interest was reported by the author.

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