Research progress of drosophila biological clock genes involved in sleep regulation

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Abstract. The normal life activities of almost all living organisms are affected by the biological clock, and the key factor affecting it is the biological clock gene. Nowly, it is basically clear the molecular regulation mechanism of the clock gene, one of the more important is the two feedback mechanism of the molecular clock, they are respectively with per gene and tim gene constitute a feedback mechanism and a feedback mechanism with clk gene as the core, they are two interdependent transcription-translation feedback mechanism. The period, timeless, clock and cycle genes that have been found and studied thoroughly in drosophila have corresponding genes or obvious homologous genes in human body, and all play an important role in sleep-related diseases. Recently, studies on sleep-related diseases have mostly involved these four clock genes and detected genetic changes in one or more of them.Among these sleep-related diseases, Alzheimer's disease (AD) and obstructive sleep apnea syndrome(OSA) have been studied more.In this paper, the discovery of drosophila clock genes, molecular regulatory mechanisms and recent studies about the relationship between the clock genes and the sleep-related diseases will be briefly reviewed,and prospects for future treatment of sleep-related diseasess will be made on this basis.

Keywords: *drosophila, biological clock gene, feedback mechanism, sleep-related diseases*.

1. Introduction

The normal life activities of almost all living organisms are affected by the biological clock, and the key factor affecting it is the biological clock gene. At present, the research on the circadian clock gene is the most comprehensive in the Drosophila body clock gene.The body clock genes include the period (per) gene, the timeless(tim) gene, the clock(clk) gene, the cycle (cyc) gene, the vrille genes. Nowadays, it is basically clear the molecular regulation mechanism of the clock gene, one of the more important is the two feedback mechanism of the molecular clock, they are respectively with per gene and tim gene constitute a feedback mechanism and a feedback mechanism with clk gene as the core, they are two interdependent transcription-translation feedback mechanism [1].

With the in-depth research of biological clock genes, it is found that biological clock catkins disorder is closely linked to Alzheimer's disease(AD), cancer and other diseases. Take AD as an example:the patients often experience lethargy, insomnia and other rhythms. Recently, more research on circadian clock genes is to explore the action mechanism of some circadian clock genes and sleep diseases. Among all the clock genes, the per, the tim, the clk and the cyc are more well studied,and the clock genes related to sleep rhythm are basically related to these four [1, 2].

This paper will discuss the development of clock gene, the molecular regulation mechanism in sleep-related diseases and the latest findings of targeted therapy based on clock gene and explore what aspects to treat sleep-related diseases and rhythm in the future.

2. Per Gene Ang Tim Gene

The per and tim are nocturnal transcription factors. The origin of these genes has been traced back more than 50 years. In the 1970s, Some scientists identified a gene regulating the rhythm in the drosophila, which called the per gene [3]. With the time going theperiod gene was successfully isolated by Michael Young team [4]. In no time, the tim was confirmed from the Drosophila chromosome by Amita Sehgal [5]. These two genes are found not only in Drosophila, but also in humans, each gene is a PER family and a TIM family composed of multiple genes.

2.1. Molecular regulation mechanism of per and tim gene

It is found that per RNA and protein is cyclically expressed, and the rise of protein level is related to the decline of mRNA level, which leads to the negative regulation of per protein's transcription,resulting in a self-regulated circadian cycle. The follow-up study of Tim supports this view, indicating that the two mRNA cycles in phase, per and tim proteins interact directly and affect their own transcription [6].

Per genes mainly transcription per mRNA, which produce PER proteins, but period genes are regulated by feedback from PER proteins, and they have cycle fluctuation characteristic. When PER protein is generated, per mRNA becomes less, that is, PER protein will inhibit period gene expression. There are still many unknowns about the regulation of the PTM about the clock proteins, including the mechanism by which phase-specific phosphorylation and O-GlCN acylation jointly regulate time function [7]. Both the period gene produces per mRNA and the subsequent PER protein is a 24h cycle of "generation-accumulation-degradation" cycle, which is consistent with the change of sleep rhythm in flies, but the output maximum value of per mRNA is earlier than the PER protein. This phenomenon may be related to the previously mentioned PER protein will inhibit period gene expression, or possibly related to the time difference of transcriptional translation [6-8].

The timeless gene, similar to the period gene, regulates the rhythm cycle through the resulting TIM protein, which is also characterized by cycle fluctuations and the same as changes in sleep rhythm in Drosophila. Both the per mRNA and the mRNA of the tim rises during the daytime and they are the highest in the time of the evening. Studies have shown that TIM proteins are susceptible to photodegradation, which reasonably explains that TIM proteins are more abundant at night and less abundant during the day, and this will also be closely related to the subsequent per/tim feedback loop, which will also be an important link between the biological clock genes and the external environment [9]. Tim gene mainly assists per gene, so that to regulate sleep rhythm.

2.2. The per / tim feedback loop

The period and timeless genes have an interactive feedback loop,in that the per and the tim proteins generated by period and timeless genes bind to each other and then feed back to the period and timeless genes and inhibit their action.Per can be stabilized by the tim in cytoplasm and then it needs to be transported to nucleus. The two proteins’s nuclear localization are also regulated by some specific imported proteins. TIM proteins can associate with PER proteins to form a heterodimeric complex that crosses the nuclear pore into the nucleus to inhibit the period gene and timeless gene action [6, 9]. Tim proteins degrade with the appearance of light, making PER proteins,and period and timeless genes will not be inhibited, thus realizing the process of "generation-accumulation-degradation" of TIM proteins, PER proteins and their associated mRNA [10].

3. The clk gene and the cyc gene

CLK and CYC are daytime transcription factors. Professor Takahashi's team first discovered the clock gene in the mice and determined its location on chromosomes [11]. Later, Allada's team found a Jrk gene in fruit flies, the Jrk gene can hinder the feedback loop of the period gene and the timeless gene, and the Jrk gene is also obviously homologous to the mouse clock gene, so the Jrk gene is called dClock, which is equivalent to the lock gene in fruit flies [12]. And then, the cycle gene was found in fruit flies, which is homologous to the bmal gene in mice [13]. At present, the research on the cycle gene is basically with the clock gene, and there are fewer studies on the cycle gene alone.

3.1. Molecular regulatory mechanisms of clock(clk) genes and cycle(cyc) genes

Like other circadian clock genes, the clock gene and the cycle gene also regulate sleep rhythms by encoding proteins, the clock gene produces the CLK protein, and the cycle produces the CYC protein, both of which belong to the bHLH-PAS transcription factor family, and all have cyclical volatility[14].

During the development of fly, clk can be activated in all of the cells containing the circadian clocks, however, the cells that generally lack the clock function expresses clk to produce ectopic clocks, which also require cyc, the same as canonical clock cells. At the same time, the cycle of the per and the tim mRNA were highly rhythmic during the light-dark (LD) cycle, but weakened during the constant dark (DD) cycle [15].

3.2. Feedback loops associated with clk genes and cyc genes

After the heterodimers of clik and CYC proteins enter into the nucleus, they bind to the E. box element on period gene and timeless gene, activating the transcription, thereby regulating the per/tim feedback loop. At night, the PER-TIM protein heterodimer enters the nucleus and binds to the CLK-CYC protein heterodimer, inhibiting the role of the CLK-CYC heterodimer; by the time light appears (starting during the day), the PER-TIM protein heterodimer breaks down, and the CLK-CYC protein heterodimer reacts, continuing the feedback cycle the next day (second round)[6, 9, 10, 16].

In addition, there is a feedback loop with the clk gene as the core. In the second feedback loop, the vrille gene and the Pdp 1 gene participate in the feedback regulation, in which the VRI protein produced by vrille inhibits the transcription of the clock gene, and the PDP protein transcribed by the Pdp 1 gene activates the transcription of the clock gene, and they both act with the clock gene to form the second feedback loop[17, 18]. The vri and the PAR domain protein 1ε gene (*Pdp1*ε) can also be activated by the clk and cyc. VRI and PDP1 ε Inhibit and activate the transcription of CYC, so as to enhance the robustness of interlocking feedback loop. CLOCKWORK ORANGE (CWO)is another downstream target of the clk/cyc. It cooperates with per to increase the oscillation amplitude [19].

4. Recent Studies On Association Of Clock Genes With Sleep Disroders

Sleep is a kind of spontaneous and reversible resting state which occurs periodically in organisms, and is ubiquitous in lower and higher organisms. In recent years, with the acceleration of the pace of life, sleep problems have become more and more prominent, and the disturbance of the biological clock is one of the important reasons. Drosophila, as a model organism at the forefront of biological clock research, will help to explore the relationship between circadian clock and sleep. The period, timeless, clock and cycle genes that have been found and studied thoroughly in drosophila have corresponding genes or obvious homologous genes in human body, and all play an important role in sleep-related diseases. Recently, studies on sleep-related diseases have mostly involved these four clock genes and detected genetic changes in one or more of them. Among these sleep-related diseases, AD and obstructive sleep apnea syndrome(OSA) have been studied more.

4.1. Alzheimer's disease(AD)

AD is a disease that frequently occurs in the elderly and is characterized by generalized dementia.In the early stages of AD,changes in sleep may be a sign of it. Compared with healthy people, patients with AD have a higher incidence of sleep-related diseasess, and sleep-related diseasess often occur after the onset of the disease, leading to the deterioration of the disease. Most people with AD will have more than one type of sleep disorder (25% to 66%).On the contrary, with the continuous progress of sleep disorder, it may lead to varying degrees of cognitive impairment, and eventually evolve into AD [20]. Recent studies have shown a bidirectional relationship between impaired clock function and AD. Interestingly, the disruption of the circadian genes,which was affected by thesleep-related diseases, exacerbates the damage of neuropathological in AD. Thus, it can be seen that the disorder of biological clock has an important impact on AD, but the specific impact and the molecular mechanism of which part of the problem are still being explored.

Circadian rhythm disorder is common in AD patients,which result in leading to impairment of the cognitive, even the mental symptoms and so on. Animal experiments have found that 6-month-old 3XTG-AD mice showed lower mRNA production of PER 1 and PER 2 genes and a 4-hour phase delay, and exhibited behavioral circadian rhythm disorder compared(CRD) with normal mice. At the

same time,compared with the the female mice,the expression of CRD in the male mice was more obvious, due to the regulation of estrogen on PER 1 and PER 2 genes [21]. These phenomena are at least partly related to altered to the changes in per1 and PER2 tran modes in SCN and may lead to cognitive impairment after CRD. Niu Long's team found abnormal expression of BMAL 1, Clock and CRY 1 genes in CSD (chronic sleep deprivation) mice (6-6.5 months of age), especially in AD mice, but the exact effect of CSD treatment on clock genes remains to be explored [22].

4.2. Obstructive sleep apnea syndrome(OSA)

OSA is the highly common illness, but the diagnosis of this disease is still insufficient, causing disruptions in molecular clocks and 24-hour sleep and the wake rhythms, the pressure of bolld and other processes that related to the biological. When people are with OSA, the airway can become partially or completely blocked, causing breathing to slow or stop, causing the body clock to get out of sync. This phenomenon all show that the biological clock disorder is inseparable from the study of biological clock genes.

Xie T's team found that the OSA was also associated with changes in clock genes, including the timeless gene and Bmal 1 gene, which are associated with HIF1α. The patients with OSA expressd more morning-evening variation(MEV) for HIF1α mRNA than the age-matched control patients without OSA(significantly by 23%, P=0.008). The gene expression levels decreased with the apnea hypopnea index(AHI) [24]. Some scholars included 133 OSA patients and 11 controls without OSA to observe the regulation and expression of core clock genes. The final study concluded that in controls without OSA, all the daily expression patterns of the circadian clock genes were observed, but in OSA patients, BMAL1, clock and CRY2 can not be observed.

In the severe OSA patients, the eight circadian clock genes' (for all but Per1) expression was down regulated at the midnight significantly. Cry1 and per3 were selected for independent factors of the severe OSA. The results further confirmed that when the cry1 and per3 were combined together,they can better predict the severity of the OSA(OR=0.58, 95%CI: 1.978 ~ 17.004, p=0.001) [25].

5. Discussion

Since the discovery of the per gene in the 1970s in drosophila, researchers have been studying the genes of the body clock. There are two main feedback loops. One is related to the heterodimer of PER-TIM protein and clK-Cyc protein, which is closely related to light factors, i. e. circadian rhythm and sleep rhythm. The other one, with the CLK gene as the core,mainly regulates the amplitude of circadian rhythm.

The period, timeless, clock and cycle genes that have been found and studied thoroughly in drosophila have corresponding genes or obvious homologous genes in human body, and all play an important role in sleep-related diseases, like AD, ASA and so on.If the body clock is out of whack. It may further affect other functions, such as endocrine and immune functions.

Therefore, researchers can compare the molecular mechanism of drosophila clock genes with that of human body clock genes, and use the important mechanisms involved in regulating sleep rhythms to investigate medical treatments or treatments related to sleep rhythms, or to monitor patients for certain sleep-related disorders.

Understanding the overall expression differences of clock genes is an effective measure to improve sleep-related diseases. Using clock genes as drug therapy targets to simulate normal circadian rhythm and improve different types of sleep-related diseases will be the focus and new direction of sleep regulation and the prevention and treatment of adverse cognitive disorders in the future.

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