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Winding the biological clock: A small molecule based approach

KEYWORDS

Circadian rhythms

Biological clock

Metabolic syndrome

Small molecules

Suprachiasmatic Nucleus

"A human body can think thoughts, play a piano, kill germs, remove toxins, make a baby all at once. Once it's doing that your biological rhythms are actually mirroring the symphony of the universe because you have Circadian rhythms, seasonal rhythms, tidal rhythms you know they mirror everything that is happening in the whole universe".

Michio Kaku, the famous physicist and futurist could not have said it any better. Derived from the Latin "circa diem" which translates to "approximately a day", the word circadian has garnered a lot of attention from researchers and general public alike. The circadian clock simply put is the body's own time keeping mechanism that is calibrated by the light and dark cycles in a 24-hr period. This biological clock is found in all living things irrespective of the species. Our very first understanding of this rather fascinating and accurate time keeping mechanism came from research carried out on the fruit fly (Drosophila melanogaster). The terms "biological clock" and "circadian rhythms" are oft used interchangeably. Despite the relationship between the two, they are not one and the same. Circadian rhythms are produced and controlled by the said biological clocks and play a rather pivotal role in regulating their timing. Taking cues from the environment and other factors, the genes that control the molecular structure of the biological clocks either turn on or off. The biological clock whilst controlling the circadian rhythms also determine the sleep-wake cycle, regulate the hormone release and help maintain the body temperature and metabolism besides other functions.

From Left to Right: Dr. Hall, Dr. Roshbash and Dr. Young were awarded the Nobel prize in Physiology or Medicine, 2017 for their groundbreaking discoveries on the molecular mechanisms controlling the body's circadian rhythm. Using fruit fly as a model, the three Nobel laureates isolated a gene that controls the daily biological rhythm in sync with the earth's revolutions.

The health of a human being is largely governed by their habits. Regular sleep and diet play a crucial role in preventing chronic disorders such as obesity, diabetes, depression and seasonal affective disorders. Researchers have already described the negative impact circadian rhythm disruption has on the human health and have identified molecular targets of small-molecule biological clock modulators. Our understanding of the small-molecule modulators needs to deepen for us to be able to decipher the key regulatory elements in the circadian network. The current edition of Transcomm, Prof Javed Iqbal summarizes on the "Pharmacological Modulation of Circadian Rhythm-related Metabolic Disorders using small molecule agonists". It is a sincere effort to educate the reader on the importance of the circadian rhythms and how certain small molecules can be deployed to combat metabolic disorders linked to perturbations in the biological clock.

Anand Ram Soorneedi

Prof. Javed Iqbal brings decades of experience across all major areas of the healthcare ecosystem. Prof. Iqbal is a pharmaceutical and biotechnology executive, serial entrepreneur, advisor, an educator, public speaker, investor, and a dynamic leader who has helped many organizations in the last three decades achieve their goals.

Prof. Iqbal is currently the program lead of Human health and wellbeing of Regional committee on Asia and Pacific (RCAP) of International council of Science (ICSU) based at Kuala Lumpur, Malaysia and Founder Chairman of Cosmic Therapeutics, Hyderabad. Prof. Iqbal is also a fellow IUPAC and a member of Indian Prime Minister's committee on CSIR society and is a member of Department of Science and Technology's committee on Drugs and Pharmaceuticals and sits on many other institutional advisory boards. Prof Iqbal has been a visiting fellow at several International universities and is currently on the international advisory board of medicinal chemistry journal CHEMMEDCHEM published by Wiley-VCH. Prof Iqbal has contributed significantly to the areas of medicinal chemistry, drug discovery and organic synthesis and has published more than 200 research papers and has filed 135 patents in the area of diabetes, infectious diseases, cancer, process chemistry for API's and pharmaceutical co-crystals. Two of the drugs discovered by his group at DRL underwent phase I clinical trials in Canada and UK.

Javed Iqbal graduated from Delhi University and worked as a research scientist at Ranbaxy Laboratories, New Delhi. Following his brief industrial stint, he moved to Cambridge University where he worked as an SERC post-doctoral fellow in the research group of Prof Ian Fleming, FRS. He later moved to Oxford University and worked as a research fellow with Prof J. E. Baldwin, FRS. He was a Professor at the Department of Chemistry, Indian Institute of Technology (IIT) Kanpur during 1984-99 and subsequently moved to Dr Reddy's Laboratories Ltd (DRL), Hyderabad where he served as Distinguished Research Scientist and Global Head, Discovery Chemistry during 2000-07. Prof Iqbal served as a Director of Regional Research Laboratory (CSIR) Trivandrum during 2002 and of Dr Reddy's Institute of Life Sciences, Hyderabad during 2007-2013.

Introduction: The intrinsic and genetically operated timekeeping system referred to as “circadian clock” is an essential timing system driving daily oscillations of physiology and behavior, including sleep/wake cycles, cell division cycles, metabolism, cardiovascular functions, hormone secretion, and mood balance. Circadian rhythms encompass several ubiquitous biological oscillations over 24-h period that are evolutionarily conserved from cyanobacteria to humans. This periodic rhythm is not a simple response to alternating changes of day and night rather the internal timekeeping system that allows organisms to anticipate environmental changes, thereby optimizing their physiology and behavior at the right time of day. The biological clock also greatly contributes to ensuring that certain biological processes take place in coordination with others. The circadian clock is self-sustainable by an elaborate cooperation of genetic components and most cells in multi-cellular organisms harbor their own cell-autonomous oscillators, which are hierarchically organized into a circadian timing system. At the apex of the mammalian circadian system, the suprachiasmatic nucleus (SCN) in the hypothalamus composed of densely packed neurons generates self-sustaining rhythms by both genetic and neural mechanisms and thus is considered as the central or master clock. The SCN central clock receives the environmental time information (primarily light) to adjust or entrain its phase and then orchestrates other oscillators in extra-SCN brain regions and peripheral tissues to exhibit overt circadian rhythms such as the rest-activity cycle, periodic daily variations in metabolism and body temperature, and the rhythmic secretion of hormones.

Mechanism of Circadian Rhythm: At the molecular level, the cellular oscillator is similar in both SCN and peripheral tissues, containing interlocked negative feedback loops. In the primary clock feedback loop, heterodimeric transcription factors (CLOCK/BMAL1 and NPAS2/BMAL1) drive expression of the Period1/2 and Cryptochrome1/2 genes. The encoded PER1/2 and CRY1/2 proteins in turn heterodimerize and repress CLOCK/BMAL1 and NPAS2/BMAL1 activity to inhibit their own expression. In addition, a secondary feedback loop consisting of the nuclear hormone receptors (REV–ERBs and RORs) directly regulates Bmal1 gene transcription, thus modulating the transcriptional output of the primary loop. REV-ERBs also have a key role in controlling various circadian outputs by cooperation with a variety of cell type-specific transcriptional regulators. Taken together, these two interlocked feedback loops provide a molecular basis for the self-sustaining circadian oscillations with a period of approximately 24 hours. **The post-translational regulatory mechanisms influences the circadian clock** as a wide range of auxiliary proteins such as protein kinases, chromatin modifying proteins and RNA-binding proteins are related to the control of protein stability, subcellular trafficking, and transcriptional activity of clock proteins, thereby contributing to fine and precise control of the cellular circadian rhythms. Among these posttranslational regulatory mechanisms, phosphorylation state of the negative limb proteins, PERs and CRYs is key to setting the period because phosphorylation-dependent degradation of PER and CRY proteins is required to terminate the repression phase leading to initiation of a new cycle of transcription.

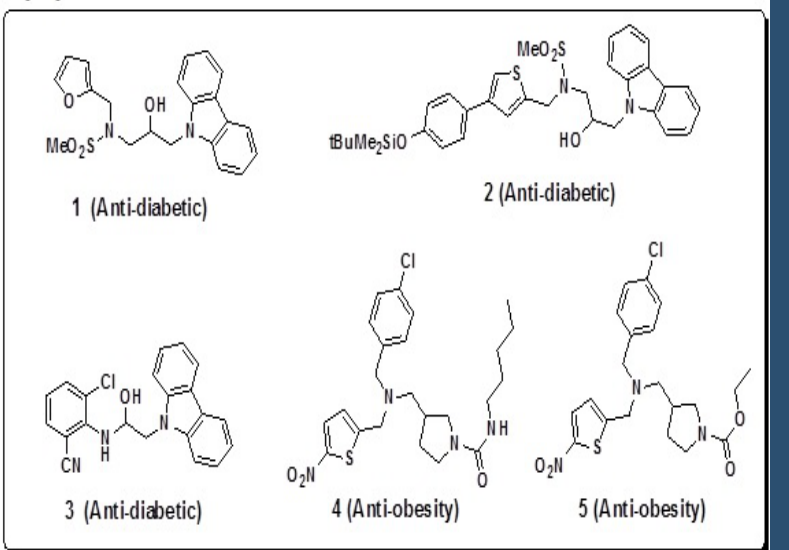
Therapeutic potential for Circadian Rhythm Related Diseases: Several research studies have shown that a robust circadian timing is prerequisite for human health and disruption of the intrinsic rhythms leads to diverse pathological states. For instance, misalignment of the intrinsic oscillators by shift-work, jetlag (either physical or social) or irregular food intake is strongly associated with various human diseases such as sleep disorders, metabolic syndrome, affective disorders and even tumorigenesis. Phenotypic analyses on mutant mice models with defective clock genes along with human genetic studies also supported the above notion and revealed mechanistic links between disrupted circadian clock and the onsets of these circadian rhythm related diseases. As a result of extensive studies on circadian clock and its functional roles in the last decades, the identification of small molecule chemical compounds capable of modulating circadian clocks either directly or indirectly has become an emerging issue. Recent studies have resulted in discovery of small chemical compounds that can pharmacologically modulate circadian timing system. The discovery of endogenous ligand for REV-ERBs led to identification of compounds which in a rest period impaired locomotor activities and during the subsequent active period significantly affected the circadian expression of core clock genes in the murine hypothalamus, indicating that these compounds sufficiently and selectively enhance REV-ERBs-mediated transcriptional repression in vivo. These small molecules have promising therapeutic potential to modulate the activity of core components of the molecular circadian clock.

Small Molecules Modulators of CRY Proteins and REV-ERBs: A Novel Therapeutic Strategy for Metabolic Syndrome

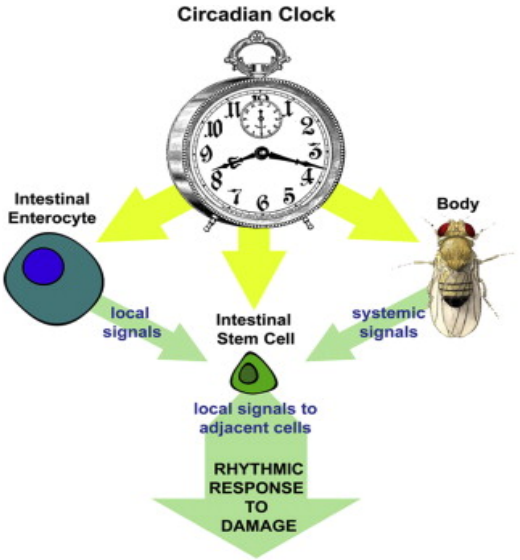
The reciprocal links between circadian clock and metabolism is well established. Thus small molecule modifiers of circadian clock have been identified for metabolic disorders as well as circadian misalignment for their therapeutic applications. The therapeutic potential of compound 1 (Figure 1), a CRYs activator/stabilizer on hepatocyte carbohydrate metabolism and diabetes has been demonstrated in mouse hepatocytes studies where CRY proteins are known to regulate fasting hormone-induced transcription of the Pck1 and G6pc genes which are associated with fasting blood glucose concentrations and type-2 diabetes in humans. Thus Compound 1 suppressed glucagon-dependent induction of Pck1 and G6pc genes without affecting their basal expression in cultured mouse primary hepatocytes and repressed glucagon-mediated activation of glucose production, suggesting the potential of 1 to control fasting hormone-induced gluconeogenesis. Two other related molecules 2 and 3 (Figure 1) were also shown to modulate the activities of CRY proteins leading to better metabolic control during circadian cycle. Recent studies have demonstrated a direct binding of compounds 1-3 (Figure 1) with CRY proteins and continuous treatment with them led to significant period lengthening and amplitude reduction of both Bmal1 and Per2 promoter activities in cultured SCN explants and fibroblast cells, implying activation of endogenous CRY proteins. It is shown that compound 1 binds to CRY protein through the FAD-binding pocket, which is known to be recognized by FBXL3 and mediate proteasomal degradation. The co-crystal structure of the compound 1 and CRY2 complex revealed that compound 1 compete with FAD to occupy the FAD binding site and then interferes with the binding of FBXL3 C-terminal to CRY, thereby stabilizing CRY proteins.

Apart from the CRYs activator, pharmacological ligands for the circadian nuclear receptors REV-ERB were also shown to modulate body metabolism in vivo. The metabolic effects of REV-ERB agonists 4 and 5 (Figure 1) were studied in mice models and chronic treatment with these agonists resulted in weight loss and reduced fat mass with increased energy expenditure. Notably, the modulation of REV-ERBs activity by agonist 4 and 5 altered daily expression of several genes related to glucose and lipid metabolism. Treatment with 4 and 5 also decreased lipogenesis and cholesterol/bile acid synthesis in the liver, increased lipid and glucose oxidation in the skeletal muscle, and decreased triglyceride synthesis. Notably, REV-ERB agonists were also effective in a high fat diet-induced obesity model as chronic treatment with compounds 4 and 5 significantly decreased plasma glucose, triglycerides, total cholesterol, non-esterified fatty acids and leptin, leading to a severe reduction in body weight and adiposity in the rodent model of obesity.

Figure 1. CRY Modulators (1-3) and REV-ERVs agonist (4 and 5) in Circadian Rythym



It conclusion, the pharmacological activation of CRYs or REV-ERBs may provide a novel therapeutic strategy to treat circadian-rhythm related disorder like obesity, metabolic and cardiovascular diseases in near future.



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