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Review Article

Structure and Function of CRY Proteins in Plant and Its Role in Cellular Processes of Human Including Cancer

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Abstract

Cryptochromes (CRY) are blue-light photoreceptors containing a Flavin Adenine Nucleotide (FAD) i.e. a type of Flavoprotein. The cryptochrome is related to DNA photolyases which is inactive in CRY of plant and animal. Two types or homologues of Cryptochromes (CRY) were first identified in plant *Arabidiopsis thaliana* as CRY1 and CRY2. Now it has been found that the genomes of most land plants contain CRY1 and CRY2. These CRY proteins are photoreceptors for blue and UV light and are involved in many biological processes of plants in controlling plant growth and development, photo-morphogenesis, circadian rhythms and others. CRYs are classified into three groups like Plant CRYs, Animal including Human CRYs and CRY-DASH proteins are found mainly in *Drosophila*. The distribution of CRYs and their structure and function have been discussed in the article. In the structure of CRYs it has been noted that there are two domains like PHR domain and C-terminal extension domain. Both are involved in mediating light signaling function for photo-morphogenesis in plant. The role of CRY proteins in plant growth, plant development has been written in the article.

The role of CRY proteins is shown in human cellular processes including circadian rhythm and its mechanism of regulating circadian clock, cell cycles, metabolism as well as the importance of circadian clocks in Cancer development and proliferation. Lastly, the therapeutic use of CRY proteins and small molecular proteins in cancer treatment has been stated.

Introduction

Cryptochromes (CRY protein) are a type of flavoprotein found in plants and animals and act mainly as blue light receptor. But these proteins are also involved in many biological processes like controlling plant growth and development, photomorphogenesis, regulating circadian rhythms etc. One special characteristics of CRY protein is that these are related with DNA photolyase. But it has lost its photolyase activity whose main function is to repair DNA damage caused by UV light. However, it has recently shown that *Arabidiopsis* CRYs can mediate blue light enhanced DNA repairing reaction (*Guo et al,* 2023; *Qu et al*, 2024). CRY proteins are generally found in green plants. Cryptochromes are classified into three groups like plant cryptochromes, animal including human cryptochromes and CRY-DASH proteins. These CRY-DASH proteins are involved in DNA repair mostly in single stranded DNA. This CRY-DASH protein has been found in Drosophila, Arabidiopsis and Human but it has also been found in photosynthetic Cyanobacteria as well as in non-photosynthetic bacteria, fungi, plants and animals. The function of CRY-DASH protein is still not very clear [1].

Cyptochromes are widely distributed in various organisms like plants, animals and bacteria having their own types but are absent in Archaea. Archaea are a group of micro-organisms like bacteria (prokaryotes) without a nucleus and other membrane bound organelles. Examples are Methanogenes, Halophiles, Thermophiles etc. So it is separate from bacteria and eukaryotes. These are thriving in extreme environments like hot springs etc.

Cryptochromes were first identified in *Arabidiopsis* where there are three types like CRY 1, CRY 2 and CRY 3. The number of cryptochromes varies in different organisms such as two in Arabidiopsis and human, six to seven in Soybean and Zebrafish [2]. Generally CRY 1 and CRY 2 have role in mediating light stimulation and photoperiodic control of floral initiation etc while CRY3 acts in repairing single stranded DNA of mitochondrial and plastid DNA. Seed plants or Spermatophytes contain two types of CRY proteins like CRY1 and CRY2. They have main function in regulating plant growth and development with response to blue light. CRY 1 ha also some specific functions in photomorphogenesis while CRY 2 is involved in photoperiodic control of flowering and is generally of nuclear protein. But CRY1 is found both in the nucleus and cytoplasm. It is known that CRY 2 has been originated from ancestor of land plants while CRY 1came from ancestor of seed plants. Land plants (Embryophytes) are those plants that live on lands while seed plants (Spermatophytes) fall under a specific group within land plants that produce seeds. So all seed plants are land plants but not all land plants are seed plants. The example of land plants are Mosses, Liverworts, Equisetum and Ferns. The example of seed plants is Angiosperms and Gymnosperms.

Cryptochromes were first identified in pant *Arabidiopsis* during the study of mutants in this plant. It has been found that hy4 mutant showed elongated hypocotyls when grown in blue light but not in other light or in the dark indicating thereby that some gene is involved in sensing the blue light. Then further research has identified another gene HY4 which encodes a protein resembling DNA-photolyase. Again, it shows that this HY4 protein binds to Flavin Adenine Nucleotide (FAD) and is lacking DNA photolyase activities leading to confirm that this protein is the Cryptochrome [3].

Structure and Function of Cryptochromes (CRY Protein)

Cryptochrome structure is generally studied through various aspects like sequence studies of cryptochromes, studies on cryptochrome mutations and on some recombinant cryptochromes. Of them sequence studies play a significant part. Sequence analysis shows that most plant CRY proteins have two domains such as i) N-terminal Photolyase Homology Region (PHR) domain which is related to DNA-photolyase and ii) but C-terminal extension (CCE domain) is not related to DNA- photolyase. In C-terminal extension additional amino acid is added to the end of C-terminus of a protein. This C-terminal extension protects the protein from degradation by proteases and gives stability to proteins to perform its role in biological systems mainly in enzymes, hormones and antibodies etc.

The PHR domain is the chromophore-binding domain and the C-terminal extension is important for the nuclear/cytosol trafficking and protein-protein interactions [4]. PHR domain is located at the N-terminal end of the protein and is highly conserved and is crucial for binding the FAD to chromophore (FAD co-factor) which is essential for light absorption and subsequent signaling. The PHR domain contains 500 amino acid region characterized by a specific- fold similar to photolyase which is important for the function of protein. The PHR regions of Cryptochromes have three-dimensional structures into two domains alpha/ beta domain and a helical domain which are connected by a loop of variable size wrapping around the alpha/beta domain. The alpha/beta domain has a dinucleotide-binding fold with 5 strand parallel Beta sheets on either side by helices. Two lobes of the helical domain form a cavity called as the FAD-access cavity in proteins. FAD binds to the protein in a U- shaped structure with its adenine and isoalloxozine rings at the bottom of cavity to bring accessible to the solvent. The PHR domain of Cryptpchromes has two chrpmophores or co-factors for absorbing light. One chromphore is FAD and the other is 5,10-methenyltetrahydrofolate (pterin or MTHF). The C-caboxy –terminal domain (CCE domain) is less conserved than the PHR region. It is present both in plant and animal cryptochromes while it is absent in CRY-DASH proteins [5]. Photolyase and CRY-DASH have a positively charged groove in the FAD access cavity where DNA binds and interacts whereas CRY 1 has no DNA-binding groove.

The PHR domain of CRY 1 and CRY2 of *Arabidiopsis* has 59% identical while C-terminal extension shows about 13% identical among them. There is a variation in the size of C-terminal extension of cryptochrmes such as about 380amino acids long in *Chlamydomonas* (algae), 181 amino acids in CRY 1 and 123 amino acids in CRY 2 of *Arabidiopsis*. But there is no C-terminal extension in white mustard Sinapis alba and fern *Adiantum capillus-veneris* [4].

It has already been stated that the PHR domain is highly conserved while the CCE domain is an intrinsically disordered region and shows higher sequence diversity than the PHR domain of CRY 1 and CRY 2. The analysis of mutants of both domains in Arabidiopsis showed that both the PHR and CCE domains are required for the function of plant CRY 1 and CRY 2. CRY 1 is involved in blue-light mediate inhibition of hypocotyls elongation and regulates some aspects of plant development . CRY 2 is responsible for regulating floral initiation and low blue light induced photomorphogenesis. CRY 1is found both in the nucleus and cytosol while CRY2 is found only in the nucleus.

Oligomerization of CRY Protein and its Function

The Oligomerization of PHR domain of CRY proteins takes place upon light absorption and so it is also known as Photo-oligomerization. It helps in the light dependent signaling and interactions with other proteins but it is a complex process involving structural changes and functional consequences. These interactions are re-

quired for regulating downstream gene expression and other physiological responses to light as well as in the light entrainment of the circadian clock in plant and non-plant species. The structural changes that occur in CRY proteins due to oligomerization takes place within the PHR domain and C-Terminal Extension (CCE) bringing the ability to enhance their light sensitivity and signaling capabilities. This process involves the formation of dimmers or large multimers which can alter the conformation of proteins and cellular components. These interactions can use light signals leading to changes in gene expression to affect developmental and physiological processes [6]. The photo-oligomerization is a photoactivation mechanism necessary for the biochemical and physiological functions of Arabidiopsis CRY 1 and CRY 2. But for the understanding of the photoactivation mechanism of CRYs, the loss-of-function mis-sense cry2 mutations were identified from 11 cry2 mis-sense mutations and were isolated and studied in detail.

It has already been stated that the oligomerization is facilitated by a light-dependent interaction between CRY photoreceptor and its signaling proteins. Plant Cryptochromes (CRY) contain Flavin Adenine Dinucleotide (FAD) chromophore that absorbs light and then it undergoes series of redox reactions leading to change in conformation of the CRY proteins, This process is accompanied by the transfer of electron from FAD to amino acid residue such as Tryptophan in the protein. The photo-excited CRY molecule leads to expose the binding sites for other proteins. It has been noted that there are four Photoregulatory Protein Kinases (PPK 1-4) which help in the phosphorylation of CRYs primarily at the CCE domain (*Qu et al,* 2024).

There are some changes of CRY proteins after photo-oligomerization. The photo-excited CRY monomers interact with each other to form dimers and tetramers for the various biological activity and protein-protein interactions. N-terminal helical subdomain of one CRY monomer interacts with the C-terminal extension (CCE) of another CRY monomer to form tetramer. The active form of CRY proteins interact with signaling proteins for gene expression and developmental processes. Oligomerization is an important step in the signaling pathways it allows CRYs to interact with other proteins involved in light dependent signaling cascades. These interactions lead to changes in gene expression in the different aspects of plant growth and development. So oligomerization is important for the light dependent functions of CRY 1 and CRY 2. CRYs can also form heterooligomers or heteropolymers. Heteropolymers are composed of different monomer types which can mimic protein-like behaviours and functions They can solubilize or stabilize proteins in non-native environments and even form proton transport channels in lipid membranes, also help in drug-delivery systems The analysis of the kinetics of the forward and reverse reaction of CRY photo-oligomerization of Arabidiopsis CRY 2 is more sensitive to blue light than CRY 1. Again, it has been noted that CRY 2 oligomers have a longer half-life in darkness than CRY 1. The study of CRY 2 photoactivation showed that CRY 2 exists as inactive monomers in the dark and the absorption of blue light makes conformational changes to make homooligopolymerization. The CRY 2 homooligomers the interact with CRY2- signaling proteins leading to regulate gene expression and photomorphogenic development [7].

In plant Cryptochromes the N-teminal domain is important for light detection and dimer formation while C-terminal extension plays an importantrole in downstream signaling pathways, interactions with other proteins and degradation in response to light particularly in Drosophila.

Transgenic Arabidiopsis seedlings expressing C-terminal extensions of CRYs fused to β- Glucouronidase (GUS) express a Constitutive Photomorphogenic (COP) phenotype indicating that the signaling mechanism of Arabidiopsis CRY is mediated through th C-terminal extension or domain. The role of the N-terminal domain of CRY is in the light activation of CRY1 photoreceptor activity [8].

Importance of Blue light Receptor CRY proteins in Plant Growth

Plants are autotrophic so they use mainly Sunlight as a source of energy to make their food through photosynthesis. Again, plants have unique mechanism to adjust growth, development and metabolic functions in the varying environments in different seasons. Plants can adjust growth under low light conditions by increasing photosynthesis and competitiveness and also can prevent oxidative damage caused by high light or UV- radiation. As plants are sessile, cannot move to a better place under adverse conditions so they have sufficient adaptive capability and enormous plasticity in growth and development for their survival resulting into the formation of different phenotypes under different environmental conditions. Plants have different types of photoreceptors for this special adaptive capacity under different environments under different light conditions. One of this important blue light photoreceptor is CRY proteins. These photoreceptors can also sense colour, direction and day-length i.e. photoperiod. There are other photoreceptors too such as Phototropins responsible for directional growth towards light (phototropism), chloroplast movement within the cell, stomatal opening and the ZEITLUPE family of receptors. Red and Far-red light are responds by phytochromes and UV-B light is sensed by the UV-B receptor giving protection from damage done by UV-light [9]. Not only sensing light CRY proteins regulate different stages of the development in plants. Different stages found in germinating seedlings like opening of embryonic leaves, differentiation of immature chloroplasts to be able to help in photosynthesis, biosynthesis of chlorophyll pigment is all done by CRYs and phytochrome photoreceptors.

Actual mechanism of activity of photoreceptor is that CRYS are accepting photons through FAD (chromophore) by binding in the PHR domain of CRYs followed by oligomerization, phosphorylation, Liquid-Liquid phase Separation (LLPS) and ubiquitation-induced degradation to transmit light signal bringing photosensitivity to

plants (*Qu et al,* 2024).

Regulation of Crys in Plant Development Through Photoresponse

The regulation done by CRYs can be clearly studied during seedling development to the induction of reproductive growth. The germinating seedling is first supported by storage foods in the seed and this process is called deetiolation where the cotyledon or embryonic leaves are open. Then it starts photosynthesis to sustain further growth. Deetiolation in light done by genes of CRYs, phytochrome -regulated genes, genes involved in photosynthesis, chloroplast formation and cell elongation. It has been found that most of these genes are under the control of the Circadian Clock. Circadian rhythms were entrained by CRYs in the day/night cycle. Phototropins directed the growth of plants towards light while CRY1 regulate the phototropic curvature of seedlings in the blue light. After the establishment of the seedling, the plant grows through meristematic cells of the Shoot Apical Meristems. The cell division of the meristematic cells has also been regulated by the light with the help of CRYs or phytochrome photoreceptors in blue and red light. When plants are growing in low light condition limiting the activity of CRYs, plants adapt a shade tolerating response by developing long petioles and lifting of leaves called Hyponysty. These CRYs can also protect plants from excess light by making the biosynthesis of substances from the oxidative damage as well as by producing anthocyanin pigments to protect from UV light [9].

Plants have Three Mechanisms in Regulating Photosensitivity such as:

i. ubiquitin E 3 Ligases Constitutive Photomorphogenic 1 (COP1) and Light response BRIC-A-BRACK/ TRAMTRACK/BROADS (LRBs) mediate degradation of photoactivated CRYs. This degradation of photoactivated CRYs is a crucial mechanism for regulating light signaling for proper photomorphogenesis.

ii. Blue Light Inhibitor of Cryptochromes 1 (BLC1) and its homologue BLC2 proteins resulting in a CRY-BLC negative feedback to control photoresponses of plants

iii. Photoactivated and Oligomerized CRYs undergo dark reversion to dissociate into inactive monomers in the absence of blue light (*Qu et al,* 2024).

It has been noted that CRYs undergo some changes in conformation after photon absorption resulting in "open" conformation to fit CRY-interacting proteins (induced fit) for photo signal transduction reminding the "Lock and Key" hypothesis of Emil Fischer in 1894. This hypothesis stated that enzyme reaction occurs when an enzyme and its substrate show complimentary geometric shapes like a **key in a lock** (*Qu et al*,2024).

It has been noted that the signal transduction is done through both PHR domain and C-Terminal Domain (CCT) in the Cryptochrome. But it has also been noted that CCT alone is sufficient to promote photomorphogenesis through some experiment in transgenic plants. Although PHR domain is needed to initiate many signaling processes. Again, transcriptome analysis of PHR and CCT transgenic lines showed that the co-regulation of CRY 1 responsive genes is done by both domains. Again, both domains of CRYs may take part in light- induced interaction with multiple signaling proteins such as COP1/SPA E3 ubiquitin ligase, many transcriptional factors, hormone signaling intermediates, proteins involved in chromatin remodeling and RNA N6 methylation [9],

Role of CRY Proteins (Cryptochromes) in Human Cellular Processes

Cryptochromes (CRY proteins) are also present in human with an important role in regulating Circadian rhythms and also sensing the magnetic field of the Earth. CRY1 and Cry2 are present in the retina and peripheral tissues and CRY 1 is highly expressed in Suprachiasmatic Nucleus (SCN) which is the central circadian pacemaker of the human body. This nucleus is present in the hypothalamus of the brain that acts as a master clock regulating circadian rhythms and it is sometimes called as Master Circadian Pacemaker. It receives light signals from the eyes and synchronizes many functions of the body with the 24hour day-night cycle. The nucleus consists of two small clusters of neurons each containing 10,000 cells. The proper function of SCN is responsible for maintaining healthy sleep patterns, hormonal balance and overall, wellbeing.

There are two types of CRY proteins such as CRY1 and CRY 2 found in the retinal ganglion cells, cone photoreceptors and in various peripheral tissues as well as in Suprachiasmatic Nucleus (SCN) regulating circadian rhythms of the body. There is also another idea that CRY proteins in human have light-sensitive magnetosensors. But the main function of CRY 1 and CRY 2 genes has a crucial role in regulating circadian rhythm or internal clock of the body. These genes along with other genes like CLOCK and BMAL1 maintain the 24-hour cycle of sleeping, waking, eating and other cellular functions of the body. It has also been noted that CRY 2 enhances the degradation of a protein called c-MYC which is a proto-oncogene that can be an oncogene if its activity is abnormally increased causing cancer.

Circadian Rhythm

The circadian rhythm or circadian clock system regulates cellular processes and behavioural processes with the external environment of 24hour day-night cycle. The main clock gene has been identified as CRY 1 which has been first identified in plant *Arabidiopsis*. This CRY protein is regulating circadian clock after binding with Flavin Adenine Dinucleotide (FAD). The gene of the CRY protein is present in the short arm of chromosome 12 of human with a length of 2.02 kb and 15 exons. The position of the CRY 1 gene I found at 12q23.3 in the short arm of chromosome 12. With the interaction of other proteins of different clock genes like PER 2 (Period 2) or BMAL1 (Brain Muscle Amt like Protein 1) CRY 1 is moved to the nucleus [10].

The circadian rhythm or internal clock in the human body is functional through an autoregulatory Transcription Translation Feedback Loop (TTFL) or it may be called transcriptional regulators for circadian Clock. It is the core mechanism driving circadian rhythms i.e., the 24-hour cycles of biological processes in living organisms helping to adapt to daily environmental cues like light and dark. The TTFL involves a series of interconnected genes whose expression is regulated by their own protein products in a feedback loop that repeats more or less every 24 hours. Further research has found two biological mechanisms such as i) the negative feedback loop of TTFL and ii) the Posttranslational Oscillation Mechanism (PTO). These two mechanisms are found in eukaryotes [11].

Mechanism of Circadian Rhythm (Clock)

The circadian clock pathway in human is very complex. The circadian clock can be divided into two such as Central clock residing in the SCN and the Peripheral clock present in every tissue and organ. The peripheral clocks can work independently of the central clock by food cues [12]. Foodcues are environmental, external and internal which stimulate a desire to eat when biologically not hungry. These cues can be external like the sight or smell of food or internal like stress and due to hormonal fluctuations. The impact of food cues can cause excessive food consumption leading to overweight and obesity. Core components for circadian clock entrainment pathways are BMAL 1 and CLOCK/NPAS 2 activating DEC, PER 1-3 and CRY 1-2 genes with the help of Transcriptional Regulators (TTFL). The circadian clock mechanism in human starts from the SCN located in the brain (master Pacemaker) after receiving light signals from the eyes through the optic nerve (detected by retinal ganglionic cells) to synchronize the 24-hour internal clock with the environment. These core clock genes mentioned above form a Transcription Translation Feedback Loop (TTFL) to form daily rhythmic oscillations in the cells of SCN and peripheral tissues. It then communicates this timing to peripheral tissues of liver, heart and endocrine glands. Then these organs along with their own self-contained molecular clocks coordinate all cellular processes of the body starting from sleep-wake cycles, hormone release, body temperature and digestion. Even in the absence of light, these SCN clocks can generate their own 24-hour rhythms although external cues are needed for precise synchronization for 24-hour cycle. The circadian proteins CLOCK (Circadian Locomotor receptor Cycles Output Kaput) and BMAL1(Brain Muscle Amt-like protein-1) heterodimerize and interact with E-Box (Enhancer Box) response elements in the promoters of target genes. These target genes are PER 1-3 and CRY 1 and 2. Other accessory proteins are DBP (D-site albumin binding protein) and the retinoid –Related Orphan Receptor (ROR) and REV-ERB through activation or repression of Bmal1 gene expression. REV-ERB is a nuclear receptor acting as a core component of the human circadian clock which regulates BMA1 and other Clock genes to maintain rhythmicity. They link the circadian system to different physiological processes like metabolism, inflammation and in Immunity through regulation of genes in the liver, adipose tissue and other organs to maintain overall health of the person.

Thus, in short, the mechanism of Circadian rhythm takes place by the primary feed-back loop through the transcription factors CLOCK and BMAL forming the positive arm of the molecular clock. These two proteins then form heterodimers for binding to cis-regulatory enhancer sequences called E-boxes (Enhancer Box) to initiate transcription of target genes (Per1-3, CRY 1 and CRY 2) to affect gene expression [13]. The CLOCK-BMAL complex have also a negative feedback effect to stabilize the regulatory loop by activating the transcription of REV-ERB and ROR to synchronize cellular function (Figure.1). So, there are two feedback loops such as

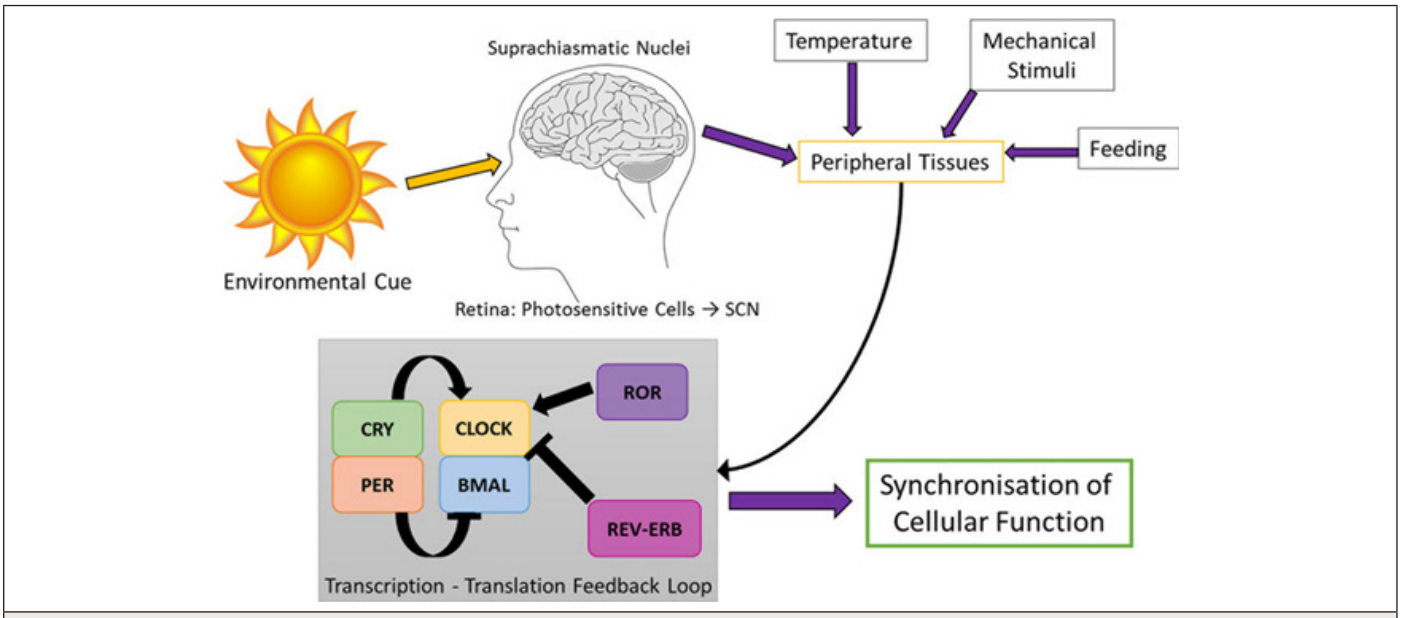

Figure 1: Showing Mechanism of Circadian Rhythm in Human. I) Light enters the SCN cells of the brain through retina via optic nerve of the eye; ii) SCN sends signals to peripheral tissues to start inner clock and again regulates it by autoregulatory feedback loop to synchronize all functions of the body [6].

a. It is known that the transcription of Per (Period) and Cry genes is induced by CLOCK/BMA1 complex to synthesize PER and CRY proteins. These proteins first accumulate in the cytoplasm shuttle between the cytoplasm and nucleus after heterodimerization. When the accumulation is high in the nucleus, they inhibit transcription of genes by blocking CLOCK/BMAL1 mediated transcriptuion. This is the first Feedback loop. And ii) the receptor (Figure 1).

REV-ERB inhibits gene expression of gene Bmal1 by binding to ROR, another receptor. This is the second feedback loop to synchronize cellular function. Circadian transcription of core clock genes is responsible for bringing rhythmicity to the expression of many genes involved in various cellular processes and metabolism. Again, the transcription of clock-controlled genes varies in different tissues i.e, tissue specific [14].

b. Cell Cycles: It is known that cell cycle is regulated by cell cycle check points so that each phase occurs in correct sequence to complete each phase (G1, S and G2) in a specific time of an organism. The important proteins controlling check points are Cyclins and CDKs (Cyclin Dependent Kinases). In G2 phase, Check point is active to prevent passing of damaged DNA to daughter cells. Cell cycle progression is controlled by the rhythmic activity of Cyclin-CDK complex. It is also known that cells are dividing without any control in Cancer cells which may be due to some mutations in cancer cells. It has also been noted that circadian clock and cell cycle are molecularly linked to regulate cell division and DNA repair processes.

It has been noted that there is an association between the expression of clock genes and cell cycle proteins through the analysis of rhythmic expression of clock genes and cell cycle proteins in human oral mucosa and skin biopsies [14,15]. But this mechanism may be dysregulated in cancer or tumour cells as circadian rhythms have been found to control many cellular processes like cell mitosis through cell cycle, expression of cell cycle proteins and the synthesis of DNA and RNA showing the importance of CRY proteins/genes and other proteins/genes regulating Circadian rhythms and Cell cycle. Mitochondrial transport signals are also located in the N-terminal portion of CRY 1 [13].

c. Metabolism

CRY 1, core component of Circadian clock, is regulating metabolism in human with the interaction of Cystathionine β-synthase (CBS) which is an enzyme in one-carbon metabolism by influencing metabolic pathways. **One Carbon metabolism** (1C) is an important biochemical process that involves the transfer of single carbon units to various molecules doing various cellular functions. It plays an important role in the synthesis of nucleotides for the synthesis of DNA and RNA, amino acids and methylation reactions for DNA and Histone modifications. One Carbon metabolism is not uniform throughout the cell. The cytoplasm, mitochondria and nucleus have their own distinct pathways and functions. Aberrant one Carbon metabolism has been implicated in the development of cancer and its progression.

In many physiological or metabolic pathways, the rate-limiting steps are very important which is controlled by circadian clock. The rate-limiting steps in a metabolic pathway is the slowest reaction that determines the overall speed of the entire pathway. These steps are sometimes catalyzed by enzymes with low maximum activity or regulated by feedback inhibition by other metabolites. [16].

CBS (Cystathionine β-synthase) is a rate limiting in the methionine cycle to regulate the methionine production. This enzyme produces cystathionine from serine and homocysteine to cystathionine. Cystathionine is then converted into cysteine by the enzyme cystathionine – γ-lyase. The first-rate activity of this enzyme is regulated by clock dynamics by enhancing the repressive activity of CRY 1 on BMAL1/CLOCK driven transcription [17].

It has also been noted that clock genes or circadian genes are responsible for metabolic syndrome. There is a link between the circadian clock and energy intake and metabolism based on changes in external factors including sunset/sunrise, physical activity and dietary intake. It has been shown through experimental studies that CRY 1 plays a major role in lipid metabolism indicating that obesity is also linked with circadian clock genes like CLOCK, BMAL1, Per 1-3, and CRY 1 and 2. The disruption of circadian rhythms may lead to many diseases like cognitive disorder, obesity, cancer, insulin resistance, metabolic syndrome etc [18].

CRY and Cancer in Human

It has been found that circadian clock/rhythm and the cell cycle are the two most important regulatory systems in eukaryotic organisms. Any disruption in the circadian rhythm through genetic or environmental factors may cause varieties of disease in human. It was known for a long time that any irregularity in cell cycle regulation is responsible for causing cancer. Recently it has been established that any disruption in circadian rhythm is increasing the risk of cancer in human. It was known that women working in night shift have significant increase in breast cancer and cancer patients with changed circadian rhythm had poorer survival rate than patients with normal circadian rhythm [19].

The epidemiologic studies were done generally in mouse model. An epidemiological study is a research method to investigate the patterns of diseases, causes and effects of health-related conditions in specific populations. It is the main discipline to understand the factors for the occurrence of diseases that can be used to prevent and control health problems. It was first noted in 1960 that environmental disruption of circadian rhythms causes breast cancer by T. Hamilton and WHO declared that the disruption in circadian rhythm is a probable carcinogen [20]. It is known that circadian rhythm is mainly controlled by SCN, located in the hypothalamus of brain, regulates daily oscillations in the production of hormones like glucocorticoids and melatonin. This level of production is altered by the disruption of circadian rhythms. Glucocorticoids are a type of steroid hormone playing a vital role in regulating various

functions of the body including metabolism, inflammation and the immune response. It has already been discussed that the main gene component of circadian rhythm is CRY 1and CRY 2.

Melatonin controls glucose homeostasis and suppresses cell and tumour growth. The production of melatonin is increase at night and decreasing during the day and is helping to synchronize circadian rhythm (internal clock) with the natural light /dark cycle. In addition to this, melatonin helps your body to get ready for sleep. Melatonin is also called chemical messenger that tells your body to relax and rest thus regulating sleep/wake cycle. It can also protect health of the brain preventing break down of brain cells. This breakdown of brain cells may lead to Dementia, Alzheimer`s and Parkinson`s diseases. Melatonin regulates glucose homeostasis which is a process that stabilizes blood glucose levels in response to changes in external and internal conditions. Glucose homeostasis and circadian rhythms are closely intertwined. Again, it is known that melatonin (produced from pineal gland of the brain) has found to inhibit tumour growth and metastasis, promotes/induces apoptosis and reduce the side effects of conventional cancer treatment. Melatonin can reduce the self-renewal capability and proliferation of cancer cells [20].

Circadian Clocks and Cancer

It has been noted the circadian clock components have some relationship with the initiation and progression of cancer This can be explained as follows:

i. It is known that Circadian Clock gene components (CRY1, CRY 2, Per 1-3) regulate the expression of several genes in different cell types leading to daily rhythms in different cellular processes like nutrient metabolism, redox regulation, autophagy, DNA-damage repair, protein folding etc. This daily rhythm is an integral part of their homeostasis. Any disruption of the circadian rhythm may disrupt these cellular processes causing a cellular environment conducive for tumourogenesis [21].

ii. There is an interaction of circadian clock proteins with other proteins for some pathways relevant to cancer showing that modulation of the expression of circadian clock proteins can protect or induce cancer.

iii. The circadian clock proteins can sense the cellular environment in some ways like change in the redox state of the cell leading to the affinity of

iv. LOCK/BMAL 1 to DNA, activity of factors regulating post translational modification of circadian clock proteins

v. Circadian clock genes regulate the expression of several secreted factors (cytokines, hormones, neurotransmitters) controlling endocrine function and signaling pathways.

The disruption of the normal function of circadian rhythm may cause different diseases in human by increasing susceptibility to development of cancer in organs, mental illness and metabolic disorders. The disruption may be due to different factors like genetic, environmental an internal disorder. The cancer may develop in breast, ovarian, lung, pancreatic, prostrate, colorectal etc. With the increase of night shift work in many countries the International Agency for Research on Cancer stated that "shiftwork that involves circadian disruption" is a probable carcinogen in 2007 [22]. Shiftwork causes disturbances in normal sleep-wake cycles that increases the risk of developing many diseases.

The sleep-wake cycle is controlled by CRY2 gene of circadian rhythm which has been identified through the study of mutant CRY 2 gene.

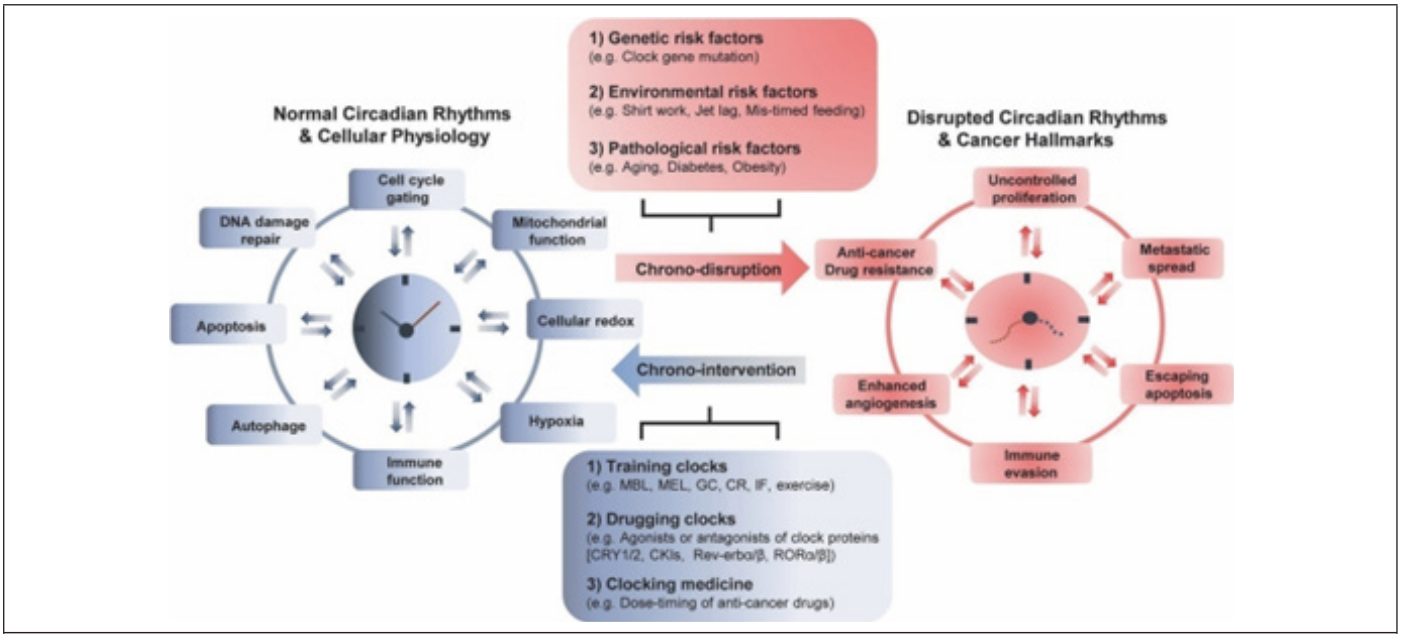

Figure 2: Showing involvement of circadian clock in cancer development and chrono-intervention after using anticancer therapy [22].

It has been noted that there is a close relation between the circadian clock and different cellular processes like cell cycle, DNA repair, apoptosis, senescence, autophagy and other oncogenic and immune pathways. So, any disorder in these processes caused by genetic, environmental and pathological risk factors etc may lead to uncontrolled cell proliferation spread of metastasis, loss of apoptosis, loss of immunity and anti-cancer drug resistance (Figure 2). Chrono-intervention or cancer intervention through surgery, chemotherapy, radiation therapy, immunotherapy can enhance or restore circadian rhythms to reduce cancer pathogenesisas well as the patient`s overall health [22] (Figure 2).

Therapeutic Use of CRY1

CRY1, protein coding gene, with length of 2.02 kb and 15 exons at 12q23.3b is found in the short arm of Human chromosome 12. CRY 1 acts as a protumorogenic factor, by rhythmically modulating DNA repair, promoting cancer cell survival. This role can be utilized as a potential therapeutic target to reduce cancer growth and increased apoptosis. It has been noted that CRY1 can increase the degradation of p53 by binding to the E3 ubiquitin ligase MDM2 in cancer cells of bladder leading to the increase in sensitivity to anticancer drugs.

Again, the anti-tumour efficiency of Cry1 has been found as CRY1 is closely related with homeostasis maintenance and tumour immunity. CRY1 has a double role both in the development of tumours as well as its function as a therapeutic target that can be used for the treatment of cancers [6].

In another study it has been noted that the combination of antiangiogenic medications and CRY1 inhibitors may have a great role in therapeutic use in HR (Homologous Recombination) deficient Epithelial ovarian cancer along with PARP (Poly ADP-Ribose polymerase) inhibitors (Lida et al 2024; [6]. It has been shown that variation in CRY1 gene is developing delayed sleep-wake phase disorder where early detection may prevent comorbidities from mood disorder, depression and Attention Deficit Hyperactivity Disorder (ADHD). Again, the it has been accepted that the over expression of CRY1 gene may be another therapeutic target as well as can be used as a biomarker for prognosis and responsiveness during chemoradiation in patients with cervical cancer [6]. Thus, the dual function of CRY1 as promoting tumour is done by the accumulation of c-myc via P13K pathway as well as by inhibiting bladder cancer through degradation of p53 with the interaction of MDM2.

Glioblastoma or Glioma is a type of cancer first started in the glial cells which are the supportive cells in the brain and spinal cord that protect nerve cells. Tumours either benign or malignant may grow in the glial cells causing problems with balance or movement. It has been noted that the small molecule modulator SHP656, may be used as a therapeutic treatment of glioblastoma and other circadian clock-related diseases as it binds to CRY2 protein and can regulate Glioblastoma Stem Cell growth and survival. The small molecule SHP656 may be an isomer of CRY2. It has been found that Giioblastoma is generally resistant to chemo and radiation therapy so this CRY2 –SHP656 complex can be a potential therapeutic agent [23].

The use of small molecules has a great potential in cancer therapy. Small molecules are low molecular weight synthetic compounds created in the laboratory that form a targeted cancer therapy by targeting specific proteins, enzymes and specific pathways for inhibiting cancer cell growth to increase survival rate. These small molecules are tyrosine kinase inhibitor like Imatinib for chronic myeloid leukemia and proteasome inhibitors like Bortezomib for multiple myelomia [24].

Use of Latest Drugs in Cancer Treatment (Targeted Therapy)

With the advancement of technology, molecular biology, Structural Chemistry, Drug design and others snall molecule anti-cancer drugs have been developed. With the introduction of first small molecule drug the Tyrosine kinase inhibitor IMATINIB in 2001 (approved by US Food and Drug Administration), several small molecule anti-cancer drugs about 89 of different types of inhibitors have been approve till December 2020 by the US Food and Drug Administration (FDA) and National Medical Products Administration (NMPA) of China [24]. The conventional method of treatmet of cancer is generally Chemotherapy, Radiotherapy and Surgical operations. One disadvantage of conventional treatment is that it cannot target cancer cells from normal cells causing death of normal cells leading to many side effects and low survival rate. The use of recent targeted drugs can specifically target cancer cells sparing normal cells leading to low toxicity and high potency. Large numbers of targeted drug are already in clinical trials for cancer treatment with a hope of getting a specific drug for specific cancer. Still there is one disadvantage of this targeted drug is to develop drug resistance in some cases.

Another trend of cancer research is now going on to find out the Epigenetic Inhibitors. This branch of Genetics studies the heritable changes of gene expression without any change in the nucleotide sequence of DNA. Epigenetic changes have also been found to cause many diseases including cancer without causing any gene mutation. Further research is going on to identify epigenetic inhibitors or epigenetic regulatory proteins that can be used as new potential targeted drugs for cancer treatment.

Again, this disruption also shows poor performance in the treatment of cancer and early mortality. However, many people follow a lifestyle that has an effect of people of shiftwork in eating a large amount of calories late at night bringing more cancer risk. Disruption of circadian rhythm causing irregularities in circadian genes causing human cancer is associated with the activation of intracellular inflammatory and onogenic signaling pathways leading to the activation of p38, c-Myc, NF-kB, BCL-XL and protein kinase A. The deregulation of molecular clock is correlated with the loss of control in cell proliferation, metabolism, DNA replication and repair and apoptosis [22].

Disruptions of the circadian rhythm can negatively impact glucose homeostasis and increase the risk of developing diabetes. This disruption may also cause tumorogenesis and facilitates the establishment of cancer. The detailed study of circadian biology in cancer research may develop a new way of prevention, diagnosis and effective way of cancer treatment.

Acknowledgment

None.

Conflict of Interest

None.

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