Retinoic acid-related orphan receptor RORβ, circadian rhythm abnormalities and tumorigenesis (Review)

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Abstract. Nuclear receptors are a superfamily of transcription factors including the steroid hormone receptors, non-steroid hormone receptors and the orphan nuclear receptor family. Retinoic acid-related orphan receptor (ROR)β, as a member of the orphan nuclear receptor family, plays an important regulatory role in the maintenance of a variety of physiological and pathological processes. RORβ has been determined to act as an osteogenic repressor in regulating bone formation, and is involved in regulating circadian rhythm. The findings of recent studies concerning the association between tumorigenesis and circadian rhythm have shown that an aberrant circadian rhythm may promote tumorigenesis and tumor progression. The mechanisms discussed in this review demonstrate how aberrant RORβ-induced circadian rhythm may become a new direction for future studies on tumorigenesis and strategy design for cancer prevention.

Contents

1. Introduction

Nuclear receptors (NRs) are a superfamily of transcription factors, which are ligand-dependent and homologous to steroid hormone receptors. NRs are widely distributed and have important physiological functions in cell development and differentiation, circadian rhythm, metabolism and immune regulation. NRs consist of three components: the steroid hormone receptors, non-steroid hormone receptors and the orphan nuclear receptor family. Steroid and non-steroid hormone receptors have specific ligands, including steroid hormones, thyroid hormones, retinoic acids and fatty acids. Ligands for orphan NRs have not yet been determined. Retinoic acid-related orphan receptors (RORs), also known as nuclear receptor subfamily 1 group F members (NR1F), are specified by gene sequences, which are homologous to retinoic acid receptor (RAR) and retinoid X receptor (RXR) which belong to non-steroid receptors (1,2). RORs include RORα, RORβ and RORγ, which are also referred to as RORA, RORB and RORC or NR1F1-3, respectively, and have been cloned from different mammalian species. The molecular mechanisms and physiological functions of RORα and RORγ are well established. However, the function of RORβ needs to be further elucidated. This review focuses on the structure of RORs and aims to provide an overview of the present studies on the functions that RORβ has in several biological and pathological processes, particularly in terms of circadian rhythm abnormalities and tumorigenesis.

2. Genotyping, molecular structures and distribution of RORs

RORα contains isoforms RORα 1-4, with only RORα1 and RORα4 being present in mice. Two isoforms of RORβ are found in mice and humans (RORβ1 and RORβ2), although studies have reported that only RORβ1 exists in humans. RORγ also has two isoforms (RORγ1 and RORγ2). RORγ2, originally found in the immune system, is often regarded as RORγt and is important in thymocyte development, with its expression being highly restricted to the thymus. The two isoforms of RORγ are found in mice and humans (3,4).

RORs share a common modular structure composed of four functional domains including an amino-terminal A/B domain, a DNA-binding domain (DBD), a hinge region and a carboxy-terminal ligand-binding domain (LBD). The A/B domains are highly variable in sequence between different ROR isoforms (5,6). The DBD consists of two highly conserved zinc finger motifs involved in the recognition of ROR response

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elements (ROREs) that contain the consensus motif AGGTCA preceded by a 5-bp AT-rich sequence. The RORs bind to ROREs as a monomer to regulate the transcription of target genes (7,8). The hinge region has shorter sequences and is thought to be a flexible domain that connects the DBD with the LBD. Its main roles are to maintain the structural stability of RORs and influence intracellular trafficking and subcellular distribution. The multifunctional domain LBD, consisting of 12 classic α helices (H1-12) and two additional α helices (H2' and H11'), plays multiple roles in ligand binding, nuclear localization, receptor dimerization, and serves as an interface for the interaction with co-activators and co-repressors (9). RORs have two activation domains, including the ligand-independent activation function-1 domain (AF-1), which is localized in the N-terminal domain and the ligand-dependent AF-2, which resides in the C-terminal domain. The transcriptional activation of AF-1 is commonly very weak, but it can synergize with AF-2 to produce a more robust upregulation of gene expression. AF-2, located in H12 and consisting of the PLYKELF motif, is 100% conserved among RORs and plays a dominant role in transcriptional regulation. The study on the crystal structure of RORα suggests that deletion or point mutation in H12, in particular Y507A, may result in a decrease in transcriptional activity and a dominant negative RORα (1).

RORs are conservative during evolution. Orthologs of RORs have been identified in some lower species, such as *Drosophila* hormone receptor 3 (DHR3) in *Drosophila melanogaster*, caenorhabditis hormone receptor 3 (CHR3) in *Caenorhabditis elegans* and manduca hormone receptor 3 (MHR3) in *Manduca sexta* (10-12). The DBDs of RORs are highly conserved and the DBD of RORγ exhibits a 92 and 75% identity with that of RORβ and RORα, respectively (13). Although the LBD sequence is moderately conserved and does not have a high degree of homology (63% for RORα and RORβ, respectively, and 58% for RORα and RORγ, respectively) (5,14), their secondary structure is very similar and always contains 12α-helices (H1-H12). H12 is 100% conserved among RORs and contains the AF2 consensus motif ΦΦXE/DΦΦ (Φ denotes a hydrophobic amino acid and X denotes any amino acid) (15), indicating that RORs may likely have similar molecular functions.

RORα is widely distributed in multiple tissues including brain, liver, pancreas, kidney, thymus, skeletal muscle, testis, ovary, lung, skin and fat tissue, and is most highly expressed in the cerebellum and hypothalamus (16-18). RORγ is mainly expressed in the thymus, kidney, skeletal muscle, heart, liver, pancreas and testis, and is particularly highly expressed in immune cells (4,19). RORβ was initially identified as a member of the orphan nuclear receptor family by Carlberg *et al* (3). It has been mapped to human chromosome 9q21.13, the *RORβ* gene, which comprises 10 exons and spans approximately a region of 188 kb of the genome (Table I). RORβ1 and RORβ2 share the common DBD and LBD, but are characterized by a different A/B domain, which, respectively, contains 2 and 13 amino acids. The N-terminal 2-13 amino acids of RORβ1 are replaced by an arginine. RORβ1 and RORβ2 consist of 459 and 470 amino acids, respectively (Fig. 1). It is likely that RORβ1 and RORβ2 originate from the same gene by either alternative splicing or transcription from an alternative promoter. RORβ was primarily detected by northern blot analysis and its expression was restricted to the central nervous system, in particular in regions involved in the modulation of circadian rhythms, such as suprachiasmatic nucleus (SCN), pineal gland, and retina (13,20-22). Recently, with the increasing sensitivity of detection, RORβ has been found outside the nervous system such as normal bone tissue, pancreatic and endometrial cancer, and uterine leiomyosarcoma (23). The expression of RORβ in normal intestinal epithelial cells and intestinal tumors has been identified using the methods of mRNA view and qPCR. In addition, intestinal tumor tissues show a lower level of RORβ when compared with paralleled adjacent intestinal tissues. In the pathological state, the expression profile of RORβ is altered, indicating that the distribution of RORβ may be more widespread than is currently known. RORβ1 and RORβ2 differ from each other as regards their distribution. RORβ1 is highly expressed in cerebral cortex layer IV, thalamus, and hypothalamus, while comparatively low expressed in retina and pineal gland. By contrast, RORβ2 is the predominant isoform in retina and pineal gland, and little is found in the cerebral areas. When compared with RORβ1, the level of RORβ2 mRNA oscillates robustly with true circadian rhythmicity and is elevated to reach its maximal value at night. RORβ1 plays a leading role in serving sensory input integration, while RORβ2 mainly affects circadian rhythmicity. The different A/B domain may contribute to their different functions.

3. Ligands identification and protein-protein interactions for RORβ

RORβ is a ligand-dependent transcription factor, although the identification of its functional ligands has not been determined. Melatonin was initially presumed as a natural ligand of RORβ due to its rhythmic synthesis and activity. RORβ can regulate circadian rhythms, and the mRNA level of RORβ coincides with melatonin production in the retina and pineal gland (22,24). However, subsequent studies have not confirmed this finding. Masana *et al* (25) found that the level of pineal melatonin exhibited a robust and significant diurnal rhythm with low levels during the day and high levels during the night in *RORβ (C3H)*$^{+/+}$, *RORβ (C3H)*$^{+/-}$ and *RORβ (C3H)*$^{-/-}$ mice, indicating that RORβ does not affect the production of pineal melatonin and melatonin is not involved in the role of RORβ-regulating circadian rhythm. In the study on the X-ray structure of LBD of RORβ, stearic acid was found to bind to RORβ and act as a filler and stabilizer rather than a functional ligand. Later studies (1,26) demonstrated that all-trans-retinoic acid (ATRA) and synthetic retinoic acid (ALRT) 1550 reversibly combined to LBD of RORβ with a high affinity, which effectively reduced the transcriptional activity mediated by GAL4-RORβ *in vitro*. They are functionally equivalent to an inverse agonist. However, the effect of ATRA on RORβ activity is cell-type-dependent. In the neuronal cell lines HT22 and Neuro2A, ATRA antagonized transactivation by RORβ, while in cells such as NIH3T3, 293, and P19, ATRA showed no effect. ATRA and ALRT 1550 are believed to be bona fide ligands for RORβ, but whether they can regulate the transcriptional activity of full-length RORβ and its target genes remains to be proven. Furthermore, ATRA and ALRT 1550 can also bind to RORγ and inhibit its transcriptional activity, but not

Table I. Characteristics of ROR isoforms.

Human chromosomal localization	Genome region (kb)	Exons	Expression	Physiological function
RORα 15q22.2	730	12	Brain, liver, lung Skin, pancreas, kidney and thymus	Brain/Purkinje cell development Bone metabolism Lymphocyte development Circadian rhythm
RORβ 9q21.3	188	10	Brain, retina, pineal gland, bone, colon, epididymus	Circadian rhythm Bone metabolism Retinal neurogenesis
RORγ 1q21.3	24	11	Thymus, kidney, heart, liver, skeletal muscle and pancreas	Lymph-node organogenesis Thymopoiesis Circadian rhythm Mesenchymal differentiation

ROR, retinoic acid-related orphan receptor.

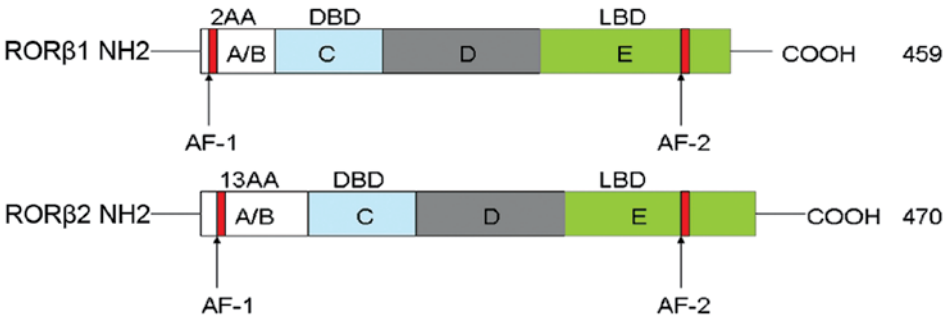

Figure 1. Comparison of the structure between RORβ1 and RORβ2. A/B, C, D and E, four functional domains. DBD, DNA-binding domain; LBD, caboxy-terminal ligand-binding domain.

to RORα. Cholesterol, identified as a putative RORα ligand, neither binds to nor modulates RORβ activity (26). Based on the finding of the crystal structure study that the AF-2 side of ligand-binding pocket (LBP) is strictly hydrophobic, it was predicted that hydrophobic molecules with a carboxylic head are more likely to be ligand candidates for RORβ (9). On the issue of synthetic ligands, significant progress has been made as regards RORα and RORγ, while little is known with regard to RORβ. Studies on its specific ligands may provide insight into our understanding of RORβ (15).

Nuclear receptor transcriptional activation domain AF-1 and AF-2 are important in protein-protein interactions (27). Co-activators such as the RNA co-activator SRA and the RNA-binding DEAD-box protein (p72/p68) interact with AF-1 (28). The co-activators acting on AF-2 including the BRG1 complex, the p160 steroid receptor co-activator (SRC) family, CBP/p300, and the TRAP/DRIP/ARC complex enhance the receptor's transcriptional activity through histone acetylation or methylation. By contrast, co-repressors such as the nuclear receptor co-repressor (NCoR) and its closely associated protein, the silencing mediator for retinoid and thyroid hormone receptor (SMRT) repress gene transcription through deacetylation by histone deacetylases (HDACs) (29). Transcriptional regulation by RORs is mediated via an interaction with co-repressors such as NCoR or co-activator complexes such as SRC-1, indicating that RORs can act as repressors and activators of transcription (30). RORβ, which contains AF-1 and AF-2, also has those molecular functions. It was also found that RORβ proteins in humans and mouse have acetylation sites K27 and K98, respectively. Thus, the regulation of target genes by RORβ is likely to be more complex.

To determine the target genes regulated by RORβ, Greiner *et al* (31) cloned a 27-kDa protein consisting of 229 amino acid residues from a cDNA library derived from mouse brain by using the method of yeast two-hybrid screen and designated it as neuronal interacting factor X1 (NIX1). NIX1 was originally identified as a neuronal-specific cofactor and is exclusively expressed in the brain including in neurons of the dentate gyrus, amygdala, thalamus, hypothalamus and several brainstem nuclei. A subgroup of NRs such as RAR and TR but not RXR or steroid hormone receptors can interact with NIX1. Furthermore, RAR and TR only interact with NIX1 in the presence of their cognate ligands, whereas NIX1 does not bind to RXR in the absence or presence of the ligand. There is no similarity of protein domains of NIX1 with other nuclear receptor cofactors except for two nuclear receptor-binding LXXLL motifs, which are also identified as nuclear location signals. One is located within the C terminus

(amino acids 192-196) and another is in an opposite orientation within the central part of the protein (amino acids 87-91) (31). Previous findings have demonstrated that LXXLL is required for the ligand-dependent binding of transcriptional co-activators to NRs (32,33). Greiner *et al* (31) found that NIX1 directly combined RORβ *in vitro* and *in vivo*, and specifically inhibited its activity in a dose-dependent manner. They also identified a minimal protein fragment spanning amino acids 61-99, which is both necessary and sufficient for the interaction between NIX1 and RORβ. Additionally, the fragment contains a nuclear receptor-binding motif in an inverted orientation (LLQAL, amino acids 87-91), which is required for the binding of NRs by NIX1. The AF-2 core motif of RORβ located at amino acid residues 445-451 is necessary for the interaction with NIX1. This finding is consistent with studies showing that elimination or deletion of AF-2 may cause the molecule to behave as a constitutive repressor or inactivate the protein (27,31,34).

NRIP2 is a protein derived from humans and it is homologous to NIX1. Compared with NIX1, NRIP2 has an extra 50 amino acids on the N terminal and is mainly distributed in the cytoplasm, which is different from NIX1. It has been found that NRIP2 and NIX1 belong to the aspartyl protease family. They are aspartyl endopeptidases and homologous to DNA damage-inducible protein (Ddi). Ddi1-related aspartyl proteases are believed to contain human homologues such as Ddi1 and Ddi2 and neuron-specific NRs NIX1, NRIP2 and NRIP3. However, the difference is that Ddi1 possesses three domains: a retroviral aspartyl-protease domain (RVP), an NH2-terminal ubiquitin-like domain (UBL), and a COOH-terminal ubiquitin-associated domain (UBA) and it is a ubiquitin receptor (35). NRIP2 lacks the three domains and contains only one conservative D-S/T-G fingerprint. The abovementioned findings suggest that NRIP2 functionally may be irrelevent to its ubiquitinated target proteins. RORβ is likely to be one of the major substrates for aspartyl endopeptidases NRIP2.

It is predicted that there are numerous interacting proteins for RORβ via UniProtKB, MINT, STRING and I2D, such as Nm23-H1(NME1), Nm23-H2(NME2), NCOA1 and MAP6. Nm23 and MAP6 are associated with cytoskeletal movement, suggesting that RORβ may be involved in the regulation of cytoskeletal movement.

4. Physiological functions of RORs

Although RORs have similar structures comprising four homologous functional domains, there are obvious differences in physiological functions, as well as in the expression between RORs. RORα, RORβ and RORγ regulate circadian rhythms and RORα plays the central role (36). RORα has a key role in the development of the cerebellum, particularly in the regulation of the maturation and survival of Purkinje cells and the formation of bone. In RORα$^{-/-}$ mice, Purkinje and granule cells are decreased and this results in cerebellar atrophy (17). RORγ is required for lymph-node organogenesis and the formation of multiple lymphoid tissues such as Peyer's patches, cryptopatches, and isolated lymphoid follicles in the intestine. RORγ$^{-/-}$ mice are deficient in lymph nodes. Moreover, RORγ promotes the differentiation of T cells and the development of lymphoid tissue-inducer (LTi) cells (37). RORβ is necessary for the proliferation and differentiation of retinal cells in addition to the maintenance of normal circadian rhythms. At birth, the retina of RORβ$^{-/-}$ mice appears very similar to that of wild-type mice with regard to morphology, but in adulthood it is disorganized and lacks the normal layer structure. The degeneration of retina occurs during the first weeks after birth and eventually results in blindness (24). RORβ$^{-/-}$ mice also exhibit behavioral changes such as reduced anxious behaviors, increased exploratory activities, changes in motor function occurring such as 'duck gait' decline in male reproductive capacity, and olfactory dysfunction (25). Recent studies have found that RORβ plays a role outside the neural system (38-41).

RORβ and retinal neurogenesis. There are two main continuous processes involved in retinal neurogenesis. One is the proliferation of retinal progenitors, which promote the growth of retina, and the other is the differentiation of the various neuronal and glial cell types that constitute the histology of the mature retina. RORβ has been found to be expressed in retinal progenitor cells but not in ganglion cells during embryonic development and it is highly expressed in the retina during embryonic and postnatal development, indicating that RORβ plays an important role in the maintenance of retinal progenitor phenotype (21) Overexpression of RORβ in retinal progenitors by biolistic transfection causes an increase in the number of large cell clones. The transcription factor Chx10, which is believed to influence retinal progenitor proliferation, is the upstream molecule of RORβ and can upregulate the transcriptional activity of RORβ. RORβ expression was markedly decreased when the genetic defect of Chx10 was present. Therefore, the role of RORβ in regulating retinal progenitor proliferation may be dependent on Chx10 (21).

RORβ is a critical transcription factor regulating rod differentiation. Rods and cones are two different types of photoreceptor cells. Rods mediate dim light vision while cones mediate daylight and color vision. The leucine zipper protein Nrl, which is restricted to be expressed in rod precursors, blocks cone differentiation when ectopically expressed in cones (42). Jia *et al* (43) reported that decreased rods and overproduced cones were observed in RORβ$^{-/-}$ mice, which were deficient in outer segments and expression of Nrl, and re-expression of Nrl in RORβ$^{-/-}$ mice converted cones to rod-like cells. As the upstream regulator of Nrl in the rod transcriptional pathway, RORβ is a key transcription factor for rod differentiation. Amacrine and horizontal cells are critical for integrating visual information. Ptf1a and Foxn4 are two early-acting factors that are essential for the generation of amacrine and horizontal cells. RORβ1 has been shown to promote the differentiation of amacrine and horizontal cells and synergistically induced expression of Ptf1a with Foxn4. Ectopic expression of RORβ1 in neonatal retina promoted amacrine cell differentiation (38).

RORβ and osteogenesis. Osteoblastic bone formation essentially involves several highly complex processes including osteoblastic differentiation, maturation and mineralization. Numerous transcription factors are involved in these processes. RORβ was identified to act as an osteogenic repressor in regulating bone formation. It is overexpressed in primary mouse and human bone tissue, especially in undifferentiated osteoblastic cultures but downregulated during osteoblastic differentiation. Roforth *et al* (44) showed that RORβ was

significantly upregulated (>50-fold) in osteoblastic precursor cells isolated from the bone marrow of aged osteoporotic mice (18-22 months old), but markedly downregulated during osteoblastic differentiation of MC3T3-E1 osteoblasts. The following mechanisms are considered to be involved when RORβ suppresses osteogenesis (39,44). i) RORβ inhibits Runx2 activity. Runx2 is the key transcription factor driving expression of the osteoblastic phenotype and its deletion in mice results in a complete deficiency of an ossified skeleton (45). The Runx2 target genes osteocalcin and osterix are reduced in mouse osteoblastic MC3T3-E1 cells, however, the exact mechanism regarding how RORβ mediates Runx2 inhibition remains to be determined. It is most likely that the protein interaction between RORβ and Runx2 inhibits normal functions of Runx2. ii) Target genes of RORβ disrupt osteoblastic extracellular matrix (ECM) production. ECM is required for the deposition of bone mineral by providing a supporting structure and its production is modulated by the activities of several growth factors and cytokines, such as transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs) (46). TGF-β inhibitor decorin (DCN) as well as the matrix gla protein (MGP), an inhibitor of bone formation by sequestrating BMP2, are upregulated in RORβ-expressing cells. These results indicate that RORβ possibly disrupt ECM production by upregulating the ECM inhibitors. iii) RORβ promotes cell proliferation by activating the mitogen-activated protein kinase (MAPK) signaling pathway.

RORβ and tumorigenesis. Relatively less evidence has been accumulated concerning the association between RORβ and tumors. In a study on 79 patients with endometrial cancer and 12 patients with stage I serous endometrial cancer, Risinger *et al* (47) analyzed the transcriptional expression profile of oligonucleotide microarray from laser microdissection on epithelial gland cells, and reported that in endometrial cancer and serous endometrial cancer, RORβ, which showed a high-level expression in the endometrium in healthy pre- or post-menopausal women, was significantly downregulated when compared with the 12 samples of healthy postmenopausal women. However, Davidson *et al* (40) found that RORβ was upregulated in the primary leiomyosarcoma of uterus. Matijevic and Pavelic (48) demonstrated that Toll-like receptor 3 (TLR3) suppressed the expression of RORβ in the metastatic pharyngeal cancer cell line Detroit 562. RORβ was upregulated by chloroquine inhibiting TLR3 expression and downregulated by siRNA silencing TLR3, suggesting that the upregulation of RORβ was TLR3-dependent. In a recent study, we observed that RORβ was decreased in colorectal cancer when compared with paralleled para-cancerous tissues, but until recently the detailed expression levels of RORβ in other tumors are still largely unelucidated.

The molecular mechanisms of how RORβ affects tumor formation and progression remain unclear. RORβ has similar functionality with RORα due to the high homology between ROR molecules. It has been identified that RORα plays a role in the regulation of the Wnt pathway; thus, RORβ may be involved in this process. The Wnt signaling pathway is closely associated with tumor growth and development, which has been evidenced in multiple tumors, such as colon, liver, gastric, lung, ovarian, and endometrial cancer. The Wnt signaling pathway includes canonical and non-canonical pathways. The canonical Wnt pathway is also known as the Wnt/β-catenin pathway. Secreted Wnt molecules such as Wnt1, Wnt3a and Wnt8 bind to the Frizzled (Fzd) and low-density receptor-related protein 5/6 (LRP5/6) co-receptor, regulate downstream TCF/LEF family gene transcription, and affect cell behavior. Under normal circumstances, most of the β-catenin participating in the cytoskeleton regulation is sequestrated in the cytoplasm by E-cadherin located on the cell membrane, and the remaining small component of cytoplasmic β-catenin binds to APC, GSK3β and Axin. In the absence of canonical Wnt signal, β-catenin forms degradable complexes with the three molecules, which activates the phosphorylation of β-catenin and leads to its degradation by Trcp ubiquitination; thus, β-catenin is maintained at a low level in the cytoplasm. In the presence of Wnt signal, Wnt molecules bind to the transmembrane receptor Fzd, which induces its combination with the intracellular protein Dsv, resulting in the inactivation of Dsv-GSK3β-APC complexes, preventing β-catenin ubiquitination and blocking its degradation. Subsequently, free β-catenin is accumulated and translocated into the nucleus and binds with the DNA-binding protein transcription factors to activate the target gene transcription. The non-canonical Wnt pathway includes Wnt/Ca^{2+} and JNK-mediated planar cell polarity pathway, which is mainly involved in cytoskeleton rearrangement and cell polarity. Wnt5a and Wnt11 can activate the Wnt/Ca^{2+} signaling pathway (49). The non-canonical Wnt pathway commonly negatively regulates the activity of the canonical Wnt pathway (50). Lee *et al* (51) found that in colorectal cancer, phosphorylated RORαS35 by PKCα showed an enhanced combination with β-catenin and was able to bind to the promoter region of β-catenin, preventing its transcription and reducing the activity of the Wnt/β-catenin pathway. Wnt5a can also increase the phosphorylation of RORα and reduce the Wnt3a-induced expression of cyclin D1 and c-Myc. RORβ and RORα contain four domains, and this homology suggests that RORβ may share similar tumor-suppressive mechanisms with RORα by inhibiting the Wnt pathway to affect tumorigenesis.

RORβ-induced circadian rhythm abnormalities and tumorigenesis. Circadian rhythms are the daily cycles of biochemistry, behavioral and physiological changes regulated by the endogenous circadian clock, which plays an important role in the physiological function and behavior of the body (52-54). A series of physiological processes including sleep, body temperature, energy metabolism, cell cycle and hormone secretion are controlled by circadian rhythms. The association between circadian rhythm abnormalities and tumorigenesis has drawn increasing attention. Circadian rhythms of mammals are mainly controlled by hypothalamic SCN and are independent of the light-sensitive system. Destruction of SCN can cause rhythm abnormalities in experimental animals and sleep disorders in patients. Circadian behaviors can be restored in SCN-ablated rodents following re-implantation of the perinatal SCN into the brain. Core clock components generally refer to the genes that are essential for the generation and regulation of circadian rhythms in individual cells and organisms, which primarily include the period and cryptochrome families. There are three subtypes of mammalian period including Per1, Per2 and Per3 and two subtypes of cryptochrome including Cry1

and Cry2. Transcription factors CLOCK and BMAL1 form a heterodimer and bind to the E-box region of the promoter of Per and Cry to promote their transcription and expression. When Per and Cry reach a certain level, their transcription mediated by BMAL1-CLOCK complexes is in turn suppressed by direct interaction. This leads to decreased levels of Per and Cry and to a new loop of activation and suppression (55,56). NPAS2, the homolog of CLOCK, can also form a heterodimer with BMAL1 and activate the transcription of circadian genes by binding to the E-box sequences (57). Previous findings suggest that histone deacetylase SIRT1 is involved in the regulation of circadian rhythms by regulating the activity of the histone acetyltransferase of CLOCK. SIRT1 is essential for circadian transcription of several core clock genes, such as *Per2*, *Cry1*, *Bmal1* and *Rorγ*. SIRT1 binds CLOCK-BMAL1 in a circadian manner and promotes the deacetylation and degradation of Per2 (58,59).

Circadian rhythm abnormalities are associated with tumorigenesis and tumor development. The IARC suggested that abnormal circadian rhythm caused by shift work was one of the major carcinogenic factors leading to human cancers. The effects of abnormal circadian rhythms on tumorigenesis have been evaluated in pilots, flight attendants, shift workers and animal experiments. Epidemiological studies have shown increased incidences of prostate cancer and acute myeloid leukemia in pilots (60,61). The incidence of endometrial cancer is much higher in women who work in shifts day and night for >20 years as compared to those who work on normal schedules (62). Similarly, night working women are also prone to breast cancer (63). It is reported that in colorectal cancer, the incidence is significantly increased in women working at night >3 days a month for ≥15 years, and the survival time of patients with regular rhythms is 5-fold higher than patients with circadian rhythm disorders (64). Keith *et al* (65) have suggested that circadian rhythm was a more important carcinogenic factor than the family history of breast cancer.

Evidence has shown that key genes that regulate circadian rhythms are aberrantly expressed in tumor tissues. Abnormal expression of the clock gene has been found in tumor cells, for instance, in breast, ovarian, endometrial and prostate cancer, while for chronic myelogenous leukemia the expression of *Per*/*Cry* gene is downregulated by the promoter methylation (60,66,67). In a recent study, it was shown that circadian genes can function as tumor-suppressor genes (68). Tumor cell proliferation was suppressed following the overexpression of Per1 or Per2 and promoted after these genes were silenced in cultured breast and prostate cancer cell lines (69,70), although the molecular mechanisms remain unclear. Genes that regulate circadian regulation are important in the regulation of cell cycle and apoptosis. Aberrant gene expression leads to gene instability, accelerates tumor cell proliferation, and thereby increases the incidence of cancer. It is believed that cancer is a circadian rhythm disorder-related disease. Filipski and Levi (71) found that modulation of the circadian rhythm played a role in the regulation of liver tumor development. SCN ablation or experimental chronic jetlag (CJL) promoted tumor growth and CJL inhibited or altered the expression of cell cycle genes and the rhythm of the clock gene in rat liver. The incidence of diethylnitrosamine-induced liver cancer was increased in jet-lagged mice and meal timing eliminated abnormal rhythms caused by CJL and retarded tumor growth.

Circadian rhythms are regulated by RORs. Clock genes including *Cry1*, *BMAL1*, *CLOCK* and *NPAS2* are identified to contain ROREs; for example, the promoter region of *BMAL1* contains two ROREs (41,72). RORα and RORγ promote the transcription of *BMAL1* by binding with ROREs, of which RORα4 shows the greatest transcriptional activation effect on BMAL1. Although there is little evidence supporting the regulatory effects of RORβ on clock genes, RORs possess structural homology and when compared with RORα and RORγ, a high expression of RORβ is intensively confined to the SCN, pineal gland, and retina, which are the major elements responsible for the regulation of circadian rhythms. The nighttime peak level of mRNA of RORβ2 has been detected in the pineal gland and retina whose expression shows a significant circadian rhythm. Moreover, RORβ$^{-/-}$ mice are endowed with circadian rhythm abnormalities (24,25). The mechanisms of how RORβ-induced circadian rhythm abnormalities promote tumorigenesis and tumor development may become a new direction for future investigations on tumor etiology.

5. Conclusion

RORβ, as an important member of orphan nuclear receptor family, plays important regulatory roles in the maintenance of a variety of physiological processes and physiological rhythms. Circadian rhythm abnormalities are increasingly being considered as a novel incentive for tumorigenesis. Therefore, to elucidate the molecular mechanisms of RORβ, regulating tumor-associated circadian rhythm abnormalities may yield important clinical benefits on effective intervention and blocking of abnormal circadian rhythm-induced tumorigenesis.

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