



Review Article

miRNA-mediated regulation of clock gene expression in men and women with colorectal cancer and possible consequences for disease management

Iveta Herichová^{*}

Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovak Republic

ARTICLE INFO

Keywords:

per2
cry2
Sex
Gender
Circadian
p53

ABSTRACT

Background: The incidence and mortality of colorectal cancer (CRC) are persistently higher in men than in women. CRC malignancy is strongly influenced by small non-coding RNAs (miRNAs). Moreover, deregulation of the circadian molecular oscillator has been associated with CRC facilitation. To analyse possible cumulative effects of the above-mentioned factors on CRC progression, we focused on functions of sex-biased miRNAs associated with the clock genes per2 and/or cry2, which are involved in the cell cycle control and DNA damage response.

Major findings: We identified miR-24, miR-92a, miR-181a, and miR-21 associated with per2 that are up-regulated in transformed colon tissue of men. miR-93, miR-17, miR-20a, and miR-24 with higher expression in males compared to females were linked to cry2. All these miRNAs possess oncogenic potential and exert their effects mainly via inhibition of the tumour suppressors phosphatase and tensin homolog (PTEN) and/or p53. Down-regulation of PTEN and p53 in men was further strengthened by inhibition of tumour suppressor per2. Oncogenic up-regulated miRNAs associated with per2 or cry2 in the transformed colon tissue of women were not detected.

Conclusion: We conclude that the cancer-promoting, sex-biased miRNAs miR-24, miR-92a, miR-181a, miR-93, miR-17, miR-20a, and miR-21 associated with clock genes per2 and/or cry2 can contribute to the sex-dependent development of CRC via inhibition of the PTEN and p53 pathways.

1. The circadian system

The circadian system generates oscillations in cells, tissues and organ functions and behaviour with a periodicity close to 24 h and synchronises these internal processes with the external environment. The most potent synchronising factor is the light (L) dark (D) cycle; however, other cyclic cues can synchronise endogenous rhythms as well [1–3].

In mammals, the circadian system consists of a central oscillator localised in the suprachiasmatic nucleus (SCN) of the hypothalamus and peripheral oscillators localised in nearly all other tissues. Under natural conditions, an organism is exposed to several synchronising factors. The sensitivity of the central and peripheral oscillators differs in respect to particular synchronising cue. Whereas the external LD cycle is the strongest synchronising factor for the central oscillator, access to food is the dominant cue for tissues involved in digestion and metabolism. When synchronising signals are disorganised, the components of the

circadian system can dissociate, potentially leading to ineffective tissue coordination. Improper timing of endogenous rhythms is frequently observed during shift work and long-distance travelling [2]. Long-term circadian deregulation can promote or worsen several pathologies, including cancer [4,5]. Whereas disruption in the circadian system contributes to cancer pathogenesis, restoration of circadian rhythmicity slows down cancer progression [4,6].

The mechanism underlying the generation of circadian oscillations is based on rhythmic expression of clock genes. In mammals there are three homologues of period genes (per1, per2, and per3) and two homologues of cryptochrome genes (cry1 and cry2). Expression of clock genes is driven by a heterodimer consisting of the transcriptional factors BMAL1 and CLOCK, which induce per and cry expression via enhancer box (E-box). The functional homolog of CLOCK is NPAS2, which can induce transcription in an alternative complex with BMAL1. The protein products of clock genes, PER and CRY, form a complex that is

Peer review under responsibility of Chang Gung University.

^{*} Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, 842 15, Bratislava, Slovak Republic.

E-mail address: herichoval@uniba.sk.

<https://doi.org/10.1016/j.bj.2024.100784>

Received 5 July 2024; Received in revised form 30 July 2024; Accepted 7 August 2024

Available online 10 August 2024

2319-4170/© 2024 The Authors. Published by Elsevier B.V. on behalf of Chang Gung University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

translocated into the nucleus and inhibits BMAL1/CLOCK-induced transcription. A 24-h time lag between the induction and repression of clock gene expression is assured by degradation of PER proteins by casein kinase 1 and other enzymes. This negative regulation is overwhelmed when the critical concentration of PER and CRY in the cytoplasm is achieved and the PER/CRY complex is translocated in to the nucleus to interact with BMAL1/CLOCK [7].

Since E-box is abundant regulatory region, the heterodimer BMAL1/CLOCK can influence expression of many genes. Rhythmic gene expression can also be induced by secondary loops attached to the basic feedback loop. Consequently, research has shown that 10%–40% of coding genes exert a rhythmic pattern and that 90 % of them are expressed in a tissue-specific manner [8–10]. Among the numerous clock-controlled genes [7,8], many contribute to cell cycle control [7].

The circadian rhythms can also be generated by phylogenetically very old redox oscillator, which does not require transcription. Redox oscillator is driven by oxidation–reduction rhythms of peroxiredoxins, which contribute to regulation of cellular redox tone by hydrogen peroxide reduction [11,12]. Redox state of cell strongly influences transcriptional feed-back loop of clock genes [3].

2. Small noncoding RNAs

In addition to environmental factors and redox potential, clock gene expression is also regulated by small non-coding RNAs (miRNAs) [13]. miRNAs are encoded by DNA sequences, and the first step of their biosynthesis is similar to that of messenger RNA (mRNA), including regulation by transcription factors. Unlike mRNA, however, miRNA does not serve as a template for protein synthesis. The rate of miRNA biosynthesis in the nucleus is determined by enzyme Drosha and its cofactor DGCR8. The mature form of miRNA is finalised in the cytoplasm by the endonuclease Dicer. Duplex of mature miRNA is then incorporated into the RNA-induced silencing complex (RISC), which contains except of Dicer also some of member of the Argonaute protein family. The RISC complex facilitates the targeting of mRNA by miRNA, which in most cases results in the inhibition of gene expression. Depending on the target genes, miRNAs can fulfil the role of tumour suppressor or oncogene [14].

2.1. Regulatory relationship of miRNAs and the circadian system

It has been shown that approximately 32% of conserved non-coding RNA exerts a rhythmic expression pattern [10]. Microarray screening of miRNAs from mouse liver tissue revealed 85 miRNAs with rhythmic pattern of expression, representing 13.3% of all miRNA sequences [15]. In humans, a daily rhythm in the level of miR-27b has been detected in leukocytes [16], and a daily rhythm in the levels of 26 out of 79 detectable miRNAs has been found in human plasma samples [17]. Moreover, level of the RNA-binding protein DGCR8, which creates a complex with Drosha during miRNA processing in the nucleus, exhibits a daily rhythm in the mRNA levels in the liver, heart, and kidney, whereas tonic expression has been observed in the SCN of rat. Accordingly, miR-30c and miR-34a show tissue-specific expression, with the most pronounced rhythmicity in the liver [18].

A regulatory relationship between miRNA signalling and the circadian system has also been documented in the opposite direction; i.e., miRNAs influence the circadian oscillator by inhibition of clock gene expression. On the basis of the rhythmic expression profiles of miR-181d versus clock gene and miR-191 versus *bmal1*, a regulatory relationship between these mRNA/miRNA pairs has been implicated [15]. miR-34a inhibits *per1* [19] and *per2* expression [20,21]. The clock gene *per2* is also targeted by the miR-192/194 cluster [22]. miR-7 [23] and miR-181d [24] have been shown to inhibit *cry2* expression, and miR-107 influences both *cry2* and clock gene expression [25]. Several of the above-mentioned miRNAs are directly involved in the progression of colorectal cancer (CRC) [26].

3. Regulation of cell cycle by clock genes

Both the miRNAs and protein products of clock genes influence the cell cycle. Screening of array datasets revealed a daily rhythm in the expression of cyclins B1, D1, D2, A1, A2, and E1. The pronounced rhythmicity was observed in the levels of genes encoding p21, p57, and p27, which fulfil the role of CDK inhibitors in the cell cycle [27–31]. Research has identified a daily rhythm in the cell cycle regulatory proteins GADD45 α , GADD45 β , and GOS2 [32]. Cell cycle control-related molecules showing a rhythmic pattern include also cyclin-dependent kinase 2 (CDK2) [30]. Some of these genes are regulated by BMAL1/CLOCK heterodimer (e.g. WEE1 and c-MYC; [28,30]), some of them are regulated by secondary circadian loops.

ChIP sequencing to identify BMAL1 and CLOCK target genes with rhythmic expression in several tissues revealed 28 clock-controlled genes, among which USP2, CDK4, TET2, and SIK1 were identified in addition to WEE1 and c-MYC [33].

The well-known molecule that mediate circadian control of the basic feedback loop to components of the cell cycle is c-MYC [30,32]. The circadian oscillator via c-MYC influences the daily expression pattern of cyclin D1, D2, D3, E1, E2, A, and B; GADD45 α , CDK1/2/4/6/7; CDC25 α ; E2F1; E2F2; TRP53; MDM2; p21; p27; and p57, and others [30,32].

Another example of the circadian feed-back loop extension is WEE1, which delays entry into mitosis by inhibiting of CDK1. WEE1 inhibits CDK2 activity. The role of WEE1 as an epigenetic modifier was recently demonstrated, as WEE1 phosphorylates histone H2B and inhibits histone transcription [34].

The circadian system is also interconnected with cell cycle control via regulation of p53 activity by PER2. Accordingly, the protein levels of p53 exhibits a distinct daily rhythm [35]. PER2 inhibits the negative regulator of p53, ubiquitin ligase MDM2, which induces degradation of p53. In this way PER2 prolongs p53 activity [36]. A possible role of PER2 driven p53 rhythmicity in modulation of DNA damage response in fibroblasts exposed to gamma radiation has been implicated [37].

4. Sex dependent expression of clock genes

The importance of sexual dimorphism, especially with respect to cancer treatment, gained increasing attention after chronotherapy was found to influence survival of patients with CRC in a sex-dependent manner [38]. In the multi-institutional clinical study EORTC-05963, combined administration of oxaliplatin (oxal), fluorouracil (FU) and leucovorin (LV) was tested. In chronomodulated therapy oxal was provided during the day and FU with LV during the night. This treatment was administered in 4-day course. Patients subjected to conventional therapy lasting 2 days, were provided with oxal and LV in the morning of the 1st day and LV during the 2nd day with FU infused in the afternoon and night during both days. Results showed that the risk of an earlier death was 38% higher in females subjected to chronomodulated therapy compared to those with conventional drug delivery. On the other hand, in males an earlier death risk decreased by 28% when drugs were provided in chronomodulated mode. These results emphasised the need to determine the most appropriate chemotherapy timing to achieve the best patient outcomes in a sex-dependent manner [38,39].

Basic research is in agreement with above mentioned observation and provides multiple examples of sex-dependent dissimilarities in the circadian system functioning [40–42]. The SCN as well as many other neural sites differ between men and women in the distribution of gonadal receptors [41]. Accordingly, the responsiveness of the circadian system to photic cues, and the regulation of the daily patterns of activity and sleep exhibits sex-dependent differences [40].

Later sexual dimorphism in clock gene expression has also been revealed [42,43]. A model based on the relative gene expression in tissues available in the Genotype-Tissue Expression Project implicated that the transverse colon is among the tissues with the highest number of rhythmically expressed genes. Interestingly, the transverse colon

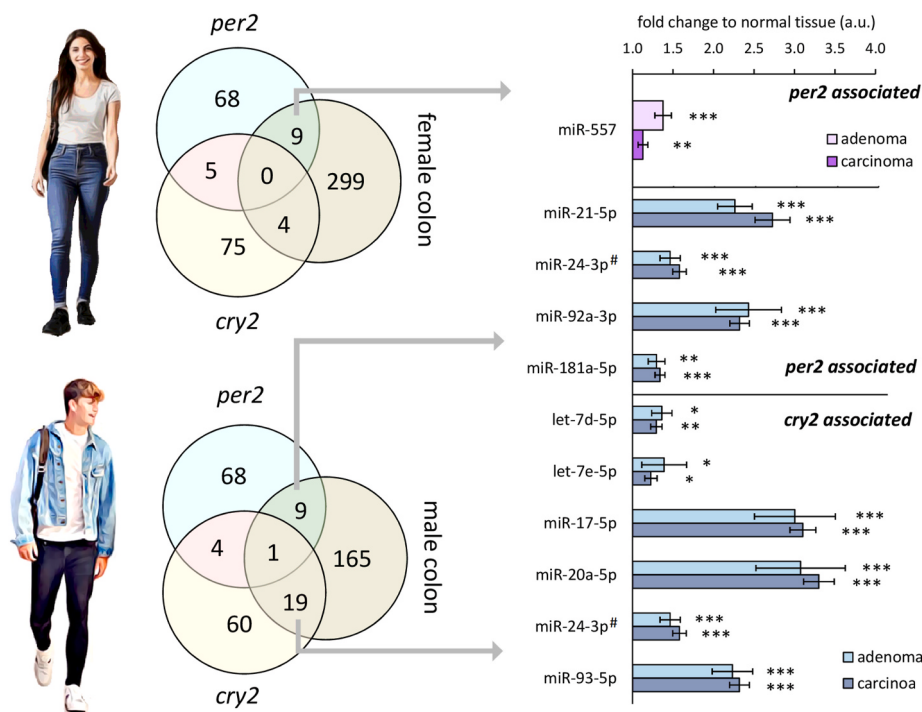


Fig. 1. Sex-biased miRNAs associated with clock genes *per2* and/or *cry2*. Left side: Venn diagram showing the overlap between miRNAs with sex-dependent expression in transformed colon tissue and miRNAs with proved functional relationship with clock genes *per2* and/or *cry2* in women (upper part) and men (lower part). Right side: Abundantly expressed sex-biased miRNAs associated with *per2* and/or *cry2* showing different expression in transformed colon tissue compared to adjacent tissue. Violet columns – women; blue columns – men. # – miRNA associated with both, *per2* and *cry2*. t-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. a.u. – arbitrary units.

contained the highest number of genes that differed in their daily expression pattern between men and women. Many more rhythmically expressed genes were identified in women than in men [44].

Deregulated expression of clock genes in tumour tissue compared with adjacent healthy tissue has been documented on many occasions; for a review see e.g. ref. [45]. In patients with CRC, *per2* and *cry2* expression was lower in tumour tissue than in adjacent healthy tissue in men, but this was not observed in women [46,47]. Therefore, effects of clock genes on cancer progression can also show sex-dependent differences.

A tumour suppressor role of the *per2* in CRC has been demonstrated in numerous in vivo and in vitro studies. For example, *per2*^{-/-} mice showed attenuated responsiveness of clock genes to gamma radiation. Similarly, the cell cycle regulators cyclin D1, cyclin A, MDM2, and GADD45α showed a deregulated pattern in *per2*^{-/-} mice that was accompanied by earlier onset of hyperplastic growth. Higher sensitivity of *per2*^{-/-} mice to gamma radiation was reflected by their lower survival relative to wild-type mice [28]. Additionally, decreased PER2 stability in *Apc*^{Min/+} mice caused deregulation of the circadian oscillator in HCT116 and SW480 cells [48]. Down-regulation of PER2 leads to prolonged AKT signalling, which is associated with resistance to chemotherapy in HCT116 cells [49]. Moreover, the *per2*^{-/-} mutation potentiated the polyp-prone phenotype of *Apc*^{Min/+} mice, and down-regulation of *per2* expression in HCT116 and SW480 cells increased both cell proliferation and the levels of β-catenin and cyclin D [50].

Unlike to *per2*, studies focusing on the role of *cry2* in CRC are less comprehensive. In spite of the lower expression of *cry2* in cancer than in adjacent tissue [46,47,51], low expression of *cry2* is usually associated with better survival in patients with CRC [47,51,52]. Silencing of *cry2* expression in DLD1 and SW480 cells decreased cell viability and increased oxaliplatin-induced apoptosis [52]. However, ectopic CRY2 increased p53 expression in HCT116 cells, decreased p53 expression in HT29 cells, and showed no effect in SW480 cells. Beyond its effect on

p53 levels, up-regulated CRY2 inhibited apoptosis in HT29, SW480, and CACO2 cell lines [51]. The tumour suppressor role of *cry2* was implicated when *cry2* silencing was found to induce proliferation of mouse embryonic fibroblasts and c-myc expression [53]. CRY2 together with FBXL3 contributes to c-MYC degradation, and in this way prevents the Warburg effect in tumour cells [24]. A recent study showed that functional CRY2 is needed for an effective p53-mediated DNA damage response [54].

5. Sex dependent expression of miRNAs

Pronounced sex-dependent differences in miRNA expression have been revealed both under physiological and pathophysiological circumstances. For example, miR-3647, miR-328, miR-1306, miR-3653, miR-1306, and miR-766 are significantly down-regulated in women with lung adenocarcinoma relative to men [55]. As extensive search of the Gene Expression Omnibus revealed 1264 female-biased and 431 male-biased miRNAs in datasets focused on infections or oncologic diseases [26].

According to our data, miR-21-5p and 3p and miR-16-5p and 3p are up-regulated in men relative to women [56]. This was also confirmed by the circulating levels of miR-21-5p and miR-16-5p in patients with CRC. In addition to miR-21 and miR-16, miR-186-5p, 22-3p, 22-5p, 25-3p, and 92a-3p were also up-regulated in men relative to women with CRC [57]. miR-30c and miR-34a exerted a more pronounced decrease in tumour tissue than in adjacent tissue in men than in women [58,59]. According to the HMDD v4.0 database [26] all the above-mentioned miRNAs are deregulated in colorectal neoplasia.

6. Possible impact of miRNA regulated clock genes on sex-dependent CRC incidence

The incidence of CRC shows a persistent sex-dependent pattern, with a higher risk of disease in men than in women [60]. An unacceptably

high number of patients diagnosed with CRC, mainly in developed countries, is persisting in spite of continual implementation of screening programmes, and the CRC burden is expected to further increase in the near future [61]. There are numerous reasons for the sex-disparity in CRC occurrence. Factors such as the body mass index, life style, diet, alcohol consumption, and smoking certainly contribute to this phenomenon [61]. In addition to the above-mentioned factors, a protective role of 17 β -estradiol has been implicated in women [62]. However, none of these factors can entirely explain the sex-biased incidence of CRC. Therefore, we focused on possible regulatory interference between miRNAs and circadian signalling, both of which are influenced by sex.

According to an analysis of the dataset GSE115513 [63] deposited in the NCBI Gene Expression Omnibus, patients with CRC exhibit pronounced sex-dependent differences in miRNA expression (all details are provided in the supplementary data files available in the Zenodo repository under the <https://zenodo.org/records/13305674>).

In healthy colon tissue, there was more men-biased miRNAs than women-biased miRNAs (34 vs. 7, respectively). This ratio was inverted in precancerous and cancerous tissues. In adenomas, the number of abundant miRNAs was 237 in woman vs. 90 miRNAs that were more expressed in men. In carcinomas, 179 sex-biased miRNAs were identified in women and 119 in men [Supp. Fig. 1].

A completely different pattern of sex-dependent miRNA expression was observed in the healthy rectal tissue, in which 27 miRNAs were more abundant in women and 5 miRNAs exerted higher expression men. The number of miRNAs showing sex-dependent expression in women decreased to three in adenoma tissues and eight in carcinoma tissues. By contrast, the number of miRNAs with higher expression increased in men to 18 in adenoma and 15 in carcinoma tissues [Supp. Fig. 1].

The list of miRNAs regulated in a sex-dependent manner was compared with the list of miRNAs functionally connected with per2 and/or cry2 expression. Information about miRNAs targeting per2 and/or cry2 was obtained from the miRTarBase database [64]. In women, nine miRNAs with sex-dependent expression were association with per2 and four miRNAs were associated with cry2. In pre- and cancerous tissues of men, 9 miRNAs were associated with per2 regulation and 19 miRNAs were associated with cry2 [Fig. 1]. miRNAs with sex-dependent expression in rectum showed minor overlap with miRNAs associated with per2 and/or cry2 expression and were excluded from further analysis (for detail see supplementary data).

miRNAs whose expression was lower than the median of a given cluster of miRNAs were also excluded from further analysis because it was unlikely that they had a strong impact on cellular processes. The selection of miRNAs with high expression is shown in Supp. Fig. 2 Supp. Fig. 3]. Expression all miRNAs with high expression showing sex-dependent up-regulation was compared between transformed and normal tissue Supp. Fig. 4 Supp. Fig. 5.

6.1. miRNAs associated with per2

Research has shown strong evidence of higher expression of miR-21 in the CRC tissue of men than in women [56,65] [Fig. 1]. miR-21 inhibits expression of the tumour suppressor PTEN [66]. A negative correlation between miR-21 and PTEN expression was detected in men but not in women according data from The Cancer Genome Atlas (Colorectal Adenocarcinoma, Nature 2012) [67].

Unlike miR-21, expression of PTEN is induced by per2. Therefore, it seems that per2 opposes the function of miR-21, which is in accordance with the generally accepted oncostatic capacity of per2 [50].

The influence of the circadian oscillator on PTEN expression is also suggested by the presence of a low-amplitude but significant daily rhythm in PTEN expression detected in some mammalian tissues, including the stomach [9,68,69]. This finding is in agreement with discovery of a conserved E-box located in the PTEN promoter. This E-box was shown to mediate induction of PTEN expression via the ubiquitous transcription factors USF1 and USF2 [70]. Whether the E-box is also

utilised by BMAL1/CLOCK remains unclear; however, a competitive interaction in signalling mediated by USF1 and BMAL1/CLOCK via the E-box was shown previously [71].

Inhibition of per2 expression by miR-21 has been implied by the miRTarBase database based on CLIP-sequencing (#MIRT655270) [64]. Similarly, according to The Cancer Genome Atlas (Colorectal Adenocarcinoma, Nature 2012) [67], there is a significant negative correlation between miR-21 and per2. Moreover, TargetScan shows that per2 is a target of miR-21 [72]. However, conclusive experimental evidence proving inhibition of per2 by miR-21 has yet to be provided.

Among miRNAs up-regulated in men [Fig. 1], miR-181a has been shown to inhibit PTEN expression in CRC tissue [73]. Inhibition of per2 by miR-181 has been suggested by the miRTarBase based on next-generation sequencing (#MIRT726984) [64] and by in vivo experiments [74]. In addition to per2, miR-181a also targets clock gene [15]. miR-181a is up-regulated in CRC tissue [75].

A member of oncomir cluster miR-17-92 and its paralogue miR-106a/363, miR-92, also inhibits the expression of PTEN [76] [Fig. 1]. Inhibition of per2 by miR-92 was demonstrated under in vitro conditions [77] and suggested by the miRTarBase (#MIRT712322). Both clusters containing miR-92 are up-regulated in CRC tissue [75].

miR-24 also targets PTEN [78,79] although this has not been demonstrated in CRC tumours. Ectopic miR-24 administration inhibits per2 expression [80].

Interestingly, the expression of another miR-21 target gene, PDCD4, which is potent tumour suppressor [81], exhibits pronounced daily rhythm in many tissues, including the SCN [9,68]. Although there are three CLOCK/BMAL1 regulatory regions in the PDCD4 gene promoter [82], a direct of E-box-mediated regulation of PDCD4 expression has yet to be provided. It has been shown that mRNA PDCD4 levels exhibit a rhythmic pattern, with an acrophase similar in 9 of 14 tissues [83].

The p53 regulatory pathway seems to be more significantly down-regulated in men than in women with CRC, particularly by miR-24 [Fig. 1]. Both tumour suppressor [84] and oncogenic potential [85] have been implicated in relation to miR-24 signalling, indicating that the effects of miR-24 depend on the biological context. However, it has also been convincingly shown that miR-24 targets p53 and in this way promotes cell survival and proliferation [86]. miR-24 also inhibits per2 expression [87,88]. Therefore, down-regulation of per2 can contribute to lower p53 activity in men in higher extend than in women with CRC [89].

p53 is targeted not only by miR-24 but also by miR-21 [90], miR-92a [91] and miR-181a (indirectly via inhibition of ATM) [92].

miR-557 has been found to be up-regulated to a greater extend in the transformed tissue of women than men [Fig. 1]. A tumour suppressor role has been attributed to miR-557 in several types of cancer via mechanisms including inhibition of Wnt/ β -catenin signalling and KRAS [93]. per2 is a target gene of miR-557 based on sequencing of RNAs linked to Argonaute proteins [93].

6.2. miRNAs associated with cry2

With respect to the up-regulated expression of miRNAs associated with cry2, we found that several members of oncomir cluster miR-17-92 [14] and its paralogue miR-106b/25 were more highly up-regulated in CRC tissue of men than of women [Fig. 1]. In particular, miR-17-92 members miR-17 and miR-20a, as well as miR-93 belonging to miR-106b/25, were up-regulated more in the male transformed gut tissue. PTEN has long been recognised as a target of miR-17-92. miR-17 induces drug resistance in CRC by PTEN inhibition [94], and miR-20a promotes CRC progression by PTEN inhibition [95]. miR-93, which shares the same seed sequence with miR-17 and miR-20a and belongs to the miR-17 family [96], also inhibits PTEN expression in CRC cell lines [97]. Therefore, it seems that PTEN expression is inhibited by both per2 and cry2-associated miRNAs. Inhibition of cry2 expression by miR-17, miR-20a, and miR-93 was implicated by miRTarBase

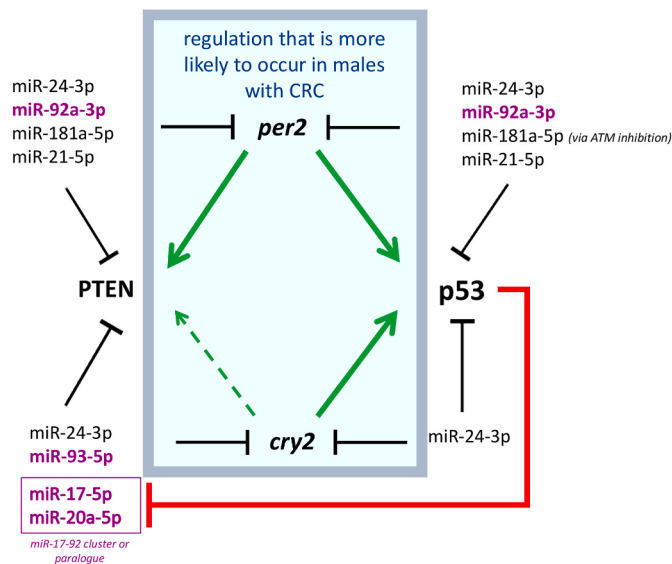


Fig. 2. Sex-dependent differences in the miRNA-regulated clock genes *per2* and *cry2* in colon cancer. Several members of oncocluster miR-17-92 and its paralogues, miR-17, miR-20a, miR-92 and miR-93, show higher expression in the transformed colon tissue of men than women. These miRNAs counteract the effects of PTEN and p53. The negative regulation by these miRNAs is further strengthened by inhibition of *per2* and/or *cry2* expression, which promote PTEN and p53 activity. Similarly, miR-21, miR-181a, and miR-24 can contribute to worse performance of men than women with CRC by interacting with the tumour suppressor *per2* and *cry2* in PTEN and p53 regulation. The solid green line shows induction, the broken green line indicates possible indirect induction, the black line implies inhibition, and the red line shows weakened inhibition. Abbreviations: CRC : colorectal cancer; PTEN : phosphatase and tensin homolog; ATR – ataxia telangiectasia mutated.

(#MIRT059185, #MIRT059186, #MIRT028006, respectively).

miR-24 is another miRNA associated with *cry2* showing higher levels in men than in women (although to a lesser extent than miR-17-92 members) [Fig. 1]. Regulation of *cry2* by miR-24 has been predicted by TargetScan and implicated by miRTarBase (#MIRT615060). However, direct evidence has not yet been provided. miR-24 targets PTEN as well as p53.

The role of *cry2* in regulation of PTEN is far less obvious than the role of *per2*. It relies mainly on the low-amplitude rhythm of PTEN expression and the presence of an E-box in PTEN promoter [9,68–70]. However, there is convincing evidence of a functional relationship between *cry2* and p53 as *cry2* mutations causes down-regulation of p53 expression [98]. p53 indirectly enhances *cry2* expression, by inhibition of miR-17-92 cluster expression [99].

Although this analysis revealed PTEN- and p53-mediated signalling as a possible regulatory interference between sex-biased miRNA and the circadian oscillator [Fig. 2], the study has several limitations. Firstly, the search was conducted based on records from miRTarBase [64], which may not contain all published experimental evidence showing regulatory relationships between miRNAs and clock genes. Moreover, most of evidence obtained from miRTarBase was achieved with use of immortalized, aneuploid cancer cell lines with unclear relevance to patient conditions. Studies were performed with the use of different methodologies and in the most cases there is only one time-point data instead of time-series that take into account circadian variations. This approach also does not include effects downstream of light, sleep, eating and lifestyle pattern that also influence the circadian system. The miRNAs identified in the study target many more genes, as noted in recent analysis. We focused on those genes that are targeted by several sex-biased miRNAs to highlight the possible cumulative impact of miRNAs that are up-regulated in men. However, as it is mentioned in particular paragraphs, in several cases validation of results in human

and/or animal species is missing.

Conclusions

It is evident that in transformed colon tissue, massive remodelling occurs in the spectrum of sex-dependent miRNAs. Based on the interactions of miRNAs with *per2* and/or *cry2* expression and functions, PTEN and p53 signalling were implicated as regulatory interferences between sex-biased miRNAs and circadian regulation. In men mostly tumour-promoting up-regulated miRNAs were detected. These miRNAs cumulatively inhibit PTEN- and p53-mediated regulation of the cell cycle. Moreover, the expression of the tumour suppressor *per2* in CRC tissue is decreased more in men than in women. We propose that knowledge about the sex-dependent partial inactivation of PTEN and p53 can contribute to targeted cancer therapy [100], designed specifically for men.

Acknowledgement

Research was supported by projects APVV-16-0209, APVV-20-0241 and VEGA 1/0455/23.

Appendix A. Supplementary data

Supplementary data to this article can be found online at DOI [10.5281/zenodo.13129792](https://doi.org/10.1016/j.bj.2024.100784) and [10.5281/zenodo.13305674](https://doi.org/10.5281/zenodo.13305674).

Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bj.2024.100784>.

References

- [1] Lévi F, Okyar A, Dulong S, Innominato PF, Clairambault J. Circadian timing in cancer treatments. *Annu Rev Pharmacol Toxicol* 2010;50:377–421.
- [2] Haus EL, Smolensky MH. Shift work and cancer risk: potential mechanistic roles of circadian disruption, light at night, and sleep deprivation. *Sleep Med Rev* 2013;17(4): 273–84.
- [3] Reddy AB. Redox and metabolic oscillations in the clockwork. In: Sassone-Corsi P, Christen Y, editors. *A time for metabolism and hormones*. Springer; 2016. p. 51–61.
- [4] Filipski E, Lévi F. Circadian disruption in experimental cancer processes. *Integr Cancer Ther* 2009;8(4):298–302.
- [5] Fortin BM, Mahieu AL, Fellows RC, Pannunzio N, Masr IS. Circadian clocks in health and disease: dissecting the roles of the biological pacemaker in cancer. *F1000Res* 2023;12:116.
- [6] Walker WH, Kaper AL, Meléndez-Fernández OH, Bumgarner JR, Liu JA, Walton JC, et al. Time-restricted feeding alters the efficiency of mammary tumor growth. *Chronobiology Int* 2022;39(4): 535–46.
- [7] Patke A, Young MW, Axelrod S. Molecular mechanisms and physiological importance of circadian rhythms. *Nat Rev Mol Cell Biol* 2020;21(2):67–84.
- [8] Lowrey PL, Takahashi JS. Mammalian circadian biology: elucidating genome-wide levels of temporal organization. *Annual Rev Genomics Hum Genet* 2004;5: 407–41.
- [9] Mure LS, Le HD, Benegiamo G, Chang MW, Rios L, Jillani N, et al. Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. *Science* 2018;359(6381):eaao0318.
- [10] Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc Natl Acad Sci U S A* 2014;111(45): 16219–24.
- [11] Ch R, Rey G, Ray S, Jha PK, Driscoll PC, Dos Santos MS, et al. Rhythmic glucose metabolism regulates the redox circadian clockwork in human red blood cells. *Nat Commun* 2021;12(1):377.
- [12] O'Neill JS, Reddy AB. Circadian clocks in human red blood cells. *Nature* 2011;469(7331):498–503.
- [13] Mendoza-Viveros L, Bouchard-Cannon P, Hegazi S, Cheng AH, Pastore S, Cheng HM. Molecular modulators of the circadian clock: lessons from flies and mice. *Cell Mol Life Sci* 2017;74(6): 1035–59.
- [14] Peng Y, Croce CM. The role of microRNAs in human cancer. *Signal Transduct Target Ther* 2016;1:15004.
- [15] Na YJ, Sung JH, Lee SC, Lee YJ, Choi YJ, Park WY, et al. Comprehensive analysis of microRNA-mRNA co-expression in circadian rhythm. *Exp Mol Med* 2009;41(9): 638–47.
- [16] Figueredo DS, Barbosa MR, Gitai DL, de Andrade TG. Predicted microRNAs for mammalian circadian rhythms. *J Biol Rhythms* 2013;28(2): 107–16.

- [17] Heegaard NH, Carlsen AL, Lilje B, Ng KL, Rønne ME, Jørgensen HL, et al. Diurnal variations of human circulating cell-free micro-RNA. *PLoS One* 2016;11(8): e0160577.
- [18] Pídková P, Chovancová B, Mravec B, Herichová I. The 24-h pattern of *dgcr8*, *drosha*, and *dicer* expression in the rat suprachiasmatic nuclei and peripheral tissues and its modulation by angiotensin II. *Gen Physiol Biophys* 2022;41(5): 417-30.
- [19] Han Y, Meng F, Venter J, Wu N, Wan Y, Standeford H, et al. MiR-34a-dependent overexpression of *Per1* decreases cholangiocarcinoma growth. *J Hepatol* 2016;64(6): 1295-304.
- [20] Olejárová S, Moravčík R, Herichová I. 2.4 GHz electromagnetic field influences the response of the circadian oscillator in the colorectal cancer cell line DLD1 to miR-34a-mediated regulation. *Int J Mol Sci* 2022;23(21):13210.
- [21] Moravčík R, Olejárová S, Zlácká J, Herichová I. Effect of miR-34a on the expression of clock and clock-controlled genes in DLD1 and Lovo human cancer cells with different backgrounds with respect to p53 functionality and 17 β -estradiol-mediated regulation. *PLoS One* 2023;18(10):e0292880.
- [22] Nagel R, Clijsters L, Agami R. The miRNA-192/194 cluster regulates the *Period* gene family and the circadian clock. *FEBS J* 2009;276(19): 5447-55.
- [23] Tang Z, Xu T, Li Y, Fei W, Yang G, Hong Y. Inhibition of *CRY2* by *STAT3*/miRNA-7-5p promotes osteoblast differentiation through upregulation of *CLOCK*/*BMAL1*/P300 expression. *Mol Ther Nucleic Acids* 2020;19: 865-76.
- [24] Guo X, Zhu Y, Hong X, Zhang M, Qiu X, Wang Z, et al. Mir-181d and c-myc-mediated inhibition of *cry2* and *fbx13* reprograms metabolism in colorectal cancer. *Cell Death Dis* 2017;8(7):e2958.
- [25] Daimiel-Ruiz L, Klett-Mingo M, Konstantinidou V, Micó V, Aranda JF, García B, et al. Dietary lipids modulate the expression of miR-107, a miRNA that regulates the circadian system. *Mol Nutr Food Res* 2015;59(3): 552-65.
- [26] Zhong B, Cui C, Cui Q. Identification and analysis of sex-biased MicroRNAs in human diseases. *Genes* 2023;14(9):1688.
- [27] Mauvoisin D, Wang J, Joffe C, Martin E, Atger F, Waridel P, et al. Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver. *Proc Natl Acad Sci U S A* 2014;111(1): 167-72.
- [28] Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian gene *Period2* plays an important role in tumor suppression and DNA damage response in vivo. *Cell* 2002;111(1):41-50.
- [29] Gréchez-Cassiau A, Rayet B, Guillaumond F, Teboul M, Delaunay F. The circadian clock component *BMAL1* is a critical regulator of p21 *WAF1*/*CIP1* expression and hepatocyte proliferation. *J Biol Chem* 2008;283(8): 4535-42.
- [30] Gaucher J, Montellier E, Sassone-Corsi P. Molecular cogs: interplay between circadian clock and cell cycle. *Trends Cell Biol* 2018;28(5): 368-79.
- [31] Griniatsos J, Michail O, Theocharis S, Arvelakis A, Papaconstantinou I, Felekouras E, et al. Circadian variation in expression of G1 phase cyclins D1 and E and cyclin-dependent kinase inhibitors p16 and p21 in human bowel mucosa. *World J Gastroenterol* 2006;12(13): 2109-14.
- [32] Soták M, Sumová A, Pácha J. Cross-talk between the circadian clock and the cell cycle in cancer. *Ann Med* 2014;46(4): 221-32.
- [33] Zhou Q, Wang R, Su Y, Wang B, Zhang Y, Qin X. The molecular circadian rhythms regulating the cell cycle. *J Cell Biochem* 2024;125(4):e30539.
- [34] Koh SB. The expanding role of *WEE1*. *Cellular Signal* 2022;94:110310.
- [35] Horiguchi M, Koyanagi S, Hamdan A, Kakimoto K, Matsunaga N, Yamashita C, et al. Rhythmic control of the ARF-MDM2 pathway by *ATF4* underlies circadian accumulation of p53 in malignant cells. *Cancer Res* 2013;73(8): 2639-49.
- [36] Gotoh T, Vila-Caballer M, Santos CS, Liu J, Yang J, Finkielstein C. The circadian factor *Period 2* modulates p53 stability and transcriptional activity in unstressed cells. *Mol Biol Cell* 2014;25(19): 3081-93.
- [37] Corrà S, Salvadori R, Bee L, Barbieri V, Mognato M. Analysis of DNA-damage response to ionizing radiation in serum-shock synchronized human fibroblasts. *Cell Biol Toxicol* 2017;33(4): 373-88.
- [38] Giacchetti S, Bjarnason G, Garufi C, Genet D, Iacobelli S, Tampellini M, et al. Phase III trial comparing 4-day chronomodulated therapy versus 2-day conventional delivery of fluorouracil, leucovorin, and oxaliplatin as first-line chemotherapy of metastatic colorectal cancer: the European Organisation for Research and Treatment of Cancer Chronotherapy group. *J Clin Oncol* 2006;24(22): 3562-9.
- [39] Lévi F, Focan C, Karaboué A, de la Valette V, Focan-Henrard D, Baron B, et al. Implications of circadian clocks for the rhythmic delivery of cancer therapeutics. *Adv Drug Deliv Rev* 2007;59(9-10): 1015-35.
- [40] Yan L, Silver R. Neuroendocrine underpinnings of sex differences in circadian timing systems. *J Steroid Biochem Mol Biol* 2016;160: 118-26.
- [41] Bailey M, Silver R. Sex differences in circadian timing systems: implications for disease. *Front Neuroendocrinol* 2014;35(1): 111-39.
- [42] Salaün C, Courvalet M, Rousseau L, Cailleux K, Breton J, Bôle-Feysot C, et al. Sex-dependent circadian alterations of both central and peripheral clock genes expression and gut-microbiota composition during activity-based anorexia in mice. *Biol Sex Differ* 2024;15(1):6.
- [43] Astafiev A, Mezhnina V, Poe A, Jiang P, Kondratov R. Sexual dimorphism of circadian liver transcriptome. *iScience* 2024;27(4):109483.
- [44] Talamanca L, Gobet C, Naef F. Sex-dimorphic and age-dependent organization of 24-hour gene expression rhythms in humans. *Science* 2023;379(6631): 478-83.
- [45] Liu H, Liu Y, Hai R, Liao W, Luo X. The role of circadian clocks in cancer: mechanisms and clinical implications. *Genes Dis* 2023;10(4): 1279-90.
- [46] Hasakova K, Vician M, Reis R, Zeman M, Herichova I. The expression of clock genes *cry1* and *cry2* in human colorectal cancer and tumor adjacent tissues correlates differently dependent on tumor location. *Neoplasma* 2018;65(6): 986-92.
- [47] Hasakova K, Vician M, Reis R, Zeman M, Herichova I. Sex-dependent correlation between survival and expression of genes related to the circadian oscillator in patients with colorectal cancer. *Chronobiol Int* 2018;35(10): 1423-34.
- [48] Yang X, Wood P, Oh E, Du-Quito J, Ansell C, Hrushesky W. Down regulation of circadian clock gene *Period 2* accelerates breast cancer growth by altering its daily growth rhythm. *Breast Cancer Res Treat* 2009;117(2): 423-31.
- [49] Yang X, He X, Yang, Jabbari E. Mammalian *PER2* regulates AKT activation and DNA damage response. *Biochem Cell Biol* 2012;90(6): 675-82.
- [50] Wood P, Yang X, Taber A, Oh E, Ansell C, Ayers S, et al. *Period 2* mutation accelerates *ApcMin/+* tumorigenesis. *Mol Cancer Res* 2008;6(11): 1786-93.
- [51] Mazzocchi G, Colangelo T, Panza A, Rubino R, De Cata A, Tiberio C, et al. Deregulated expression of cryptochrome genes in human colorectal cancer. *Mol Cancer* 2016;15:6.
- [52] Fang L, Yang Z, Zhou J, Tung J, Hsiao C, Wang L, et al. Circadian clock gene *CRY2* degradation is involved in chemoresistance of colorectal cancer. *Mol Cancer Ther* 2015;14(6): 1476-87.
- [53] Huber A, Papp S, Chan A, Henriksson E, Jordan S, Kriebs A, et al. *CRY2* and *FBXL3* cooperatively degrade c-MYC. *Mol Cell* 2016;64(4): 774-89.
- [54] Chan A, Parico G, Fribourgh J, Ibrahim J, Bollong M, Parth C, et al. *CRY2* missense mutations suppress P53 and enhance cell growth. *Proc Natl Acad Sci U S A* 2021;118(27):e2101416118.
- [55] Guo L, Zhang Q, Ma X, Wang J, Liang T. MiRNA and mRNA expression analysis reveals potential sex-biased miRNA expression. *Sci Rep* 2017;7:39812.
- [56] Hasáková K, Bezakova J, Vician M, Reis R, Zeman M, Herichova I. Gender-dependent expression of leading and passenger strand of miR-21 and miR-16 in human colorectal cancer and adjacent colonic tissues. *Physiol Res* 2017;66(Suppl 4):S575-82.
- [57] Tomeva E, Krammer U, Switzeny O, Haslberger A, Hippe B. Sex-specific miRNA differences in liquid biopsies from subjects with solid tumors and healthy controls. *Epigenomes* 2023;7(1):2.
- [58] Herichová I, Jendřisková S, Pídková P, Kršková L, Olexová L, Morová M, et al. Effect of 17 β -estradiol on the daily pattern of *ACE2*, *ADAM17*, *TPRSS2* and estradiol receptor transcription in the lungs and colon of male rats. *PLoS One* 2022;17(6):e0270609.
- [59] Hasakova K, Reis R, Vician M, Zeman M, Herichova I. Expression of miR-34a-5p is up-regulated in human colorectal cancer and correlates with survival and clock gene *PER2* expression. *PLoS One* 2019;14(10):e0224396.
- [60] Gausman V, Dornblaser D, Anand S, Hayes R, O'Connell K, Du M, et al. Risk factors associated with early-onset colorectal. *Cancer. Clin Gastroenterol Hepatol* 2020;18(12): 2752-9.
- [61] Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol* 2021;14(10):101174.
- [62] Nie X, Xie R, Tuo B. Effects of estrogen on the gastrointestinal tract. *Dig Dis Sci* 2018;63(3): 583-96.
- [63] Slattery M, Herrick J, Pellatt D, Stevens J, Mullany L, Wolff E, et al. MicroRNA profiles in colorectal carcinomas, adenomas and normal colonic mucosa: variations in miRNA expression and disease progression. *Carcinogenesis* 2016;37(3): 245-61.
- [64] Huang H, Lin Y, Cui S, Huang Y, Tang Y, Xu J, et al. miRTarBase update 2022: an informative resource for experimentally validated miRNA-target interactions. *Nucleic Acids Res* 2022;50(D1):D222-30.
- [65] Dehghan F, Boozarpour S, Torabizadeh Z, Alijanpour S. miR-21: a promising biomarker for the early detection of colon cancer. *Oncotargets Ther* 2019;12: 5601-7.
- [66] Wu Y, Song Y, Xiong Y, Wang X, Xu K, Han B, et al. MicroRNA-21 (Mir-21) promotes cell growth and invasion by repressing tumor suppressor *PTEN* in colorectal cancer. *Cell Physiol Biochem* 2017;43(3): 945-58.
- [67] Cerami E, Gao JJ, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012;2(5): 401-4.
- [68] Pizarro A, Hayer K, Lahens N, Hogenesch J. CircaDB: a database of mammalian circadian gene expression profiles. *Nucleic Acids Res* 2013;41:D1009-13.
- [69] Alibhai F, LaMarre J, Reitz C, Tsimakouridze E, Kroetsch J, Bolz S, et al. Disrupting the key circadian regulator *CLOCK* leads to age-dependent cardiovascular disease. *J Mol Cell Cardiol* 2017;105:24-37.
- [70] Grenciewicz D, Romigh T, Thacker S, Abbas A, Jaini R, Luse D, et al. Redefining the *PTEN* promoter: identification of novel upstream transcription start regions. *Hum Mol Genet* 2021;30(22): 2135-48.
- [71] Shimomura K, Kumar V, Koike N, Kim T, Chong J, Buhr E, et al. *Usl1* a suppressor of the circadian Clock mutant, reveals the nature of the DNA-binding of the *CLOCK*:*BMAL1* complex in mice. *Elife* 2013;2:e00426.
- [72] Agarwal V, Bell G, Nam J, Bartel D. Predicting effective microRNA target sites in mammalian mRNAs. *Elife* 2015;4:e05005.
- [73] Zhang X, Li X, Tan F, Yu N, Pei H. *STAT1* inhibits MiR-181a expression to suppress colorectal cancer cell proliferation through *PTEN*/akt. *J Cell Biochem* 2017;118(10): 3435-43.
- [74] Chandrasekar V, Dreyer J. Regulation of MiR-124, Let-7d, and MiR-181a in the accumbens affects the expression, extinction, and reinstatement of cocaine-induced conditioned place preference. *Neuropsychopharmacology* 2011;36(6): 1149-64.
- [75] Pídková P, Herichová I. MiRNA clusters with up-regulated expression in colorectal cancer. *Cancers* 2021;13(12):2979.
- [76] Zhang G, Zhou H, Xiao H, Liu Z, Tian H, Zhou T. MicroRNA-92a functions as an oncogene in colorectal cancer by targeting *PTEN*. *Dig Dis Sci* 2014;59(1):98-107.

- [77] Bhatwadekar A, Yan Y, Stepps V, Hazra S, Korah M, Bartelmez S, et al. miR-92a corrects CD34+ cell dysfunction in diabetes by modulating core circadian genes involved in progenitor differentiation. *Diabetes* 2015;64(12): 4226-37.
- [78] Zheng X, Li J, Peng C, Zhao J, Chi J, Meng X, et al. MicroRNA-24 induces cisplatin resistance by targeting PTEN in human tongue squamous cell carcinoma. *Oral Oncol* 2015;51(11):998–1003.
- [79] He H, Lin C, Lu Y, Wu H. Knockdown of miR-24 suppressed the tumor growth of cervical carcinoma through regulating PTEN/PI3K/AKT signaling pathway. *Biochem Genet* 2024;62(2): 1277-90.
- [80] Yoo S, Kojima S, Shimomura K, Koike N, Buhr E, Furukawa T, et al. Period2 3'-UTR and microRNA-24 regulate circadian rhythms by repressing PERIOD2 protein accumulation. *Proc Natl Acad Sci U S A* 2017;114(42):E8855–64.
- [81] Matsuhashi S, Manirujjaman M, Hamajima H, Ozaki I. Control mechanisms of the tumor suppressor PDCD4: expression and functions. *Int J Mol Sci* 2019;20(9): 2304.
- [82] Drees R, Ambrosini G, Groux R, Périer R, Bucher P. The eukaryotic promoter database in its 30th year: focus on non-vertebrate organisms. *Nucleic Acids Res* 2017;45(D1):D51–5.
- [83] Yan J, Wang H, Liu Y, Shao C. Analysis of gene regulatory networks in the mammalian circadian rhythm. *PLoS Comput Biol* 2008;4(10):e1000193.
- [84] Gao Y, Liu Y, Du L, Li J, Qu A, Zhang X, et al. Down-regulation of miR-24-3p in colorectal cancer is associated with malignant behavior. *Med Oncol* 2015;32(1): 362.
- [85] Kerimis D, Kontos C, Christodoulou S, Papadopoulos I, Scorilas A. Elevated expression of miR-24-3p is a potentially adverse prognostic factor in colorectal adenocarcinoma. *Clin Biochem* 2017;50(6): 285-92.
- [86] Chen L, Luo L, Chen W, Xu H, Chen F, Chen L, et al. MicroRNA-24 increases hepatocellular carcinoma cell metastasis and invasion by targeting p53: miR-24 targeted p53. *Biomed Pharmacother* 2016;84: 1113-8.
- [87] Park I, Kim D, Kim J, Jang S, Choi M, Choe H, et al. microRNA-25 as a novel modulator of circadian Period2 gene oscillation. *Exp Mol Med* 2020;52(9): 1614-26.
- [88] Yoo S, Kojima S, Shimomura K, Koike N, Buhr E, Furukawa T, et al. Period2 3'-UTR and microRNA-24 regulate circadian rhythms by repressing PERIOD2 protein accumulation. *Proc Natl Acad Sci U S A* 2017;114(42):E8855–64.
- [89] Haupt S, Caramia F, Herschtal A, Soussi T, Lozano G, Chen H, et al. Identification of cancer sex-disparity in the functional integrity of p53 and its X chromosome network. *Nat Commun* 2019;10(1):5385.
- [90] Papagiannakopoulos T, Shapiro A, Kosik K. MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. *Cancer Res* 2008;68(19): 8164-72.
- [91] Tao X, Zhang X, Sun S, Wu D. MiR-92a contributes to cell proliferation, apoptosis and doxorubicin chemosensitivity in gastric carcinoma cells. *Oncol Rep* 2019;42(1): 313-20.
- [92] Braicu C, Gulei D, Raduly L, Harangus A, Rusu A. Altered expression of miR-181 affects cell fate and targets drug resistance-related mechanisms. *Mol Aspects Med* 2019;70:90–105.
- [93] Cheng X, Wu C, Xu H, Zou R, Li T, Ye S. miR-557 inhibits hepatocellular carcinoma progression through Wnt/p-catenin signaling pathway by targeting RAB10. *Aging* 2024;16(4): 3716-33.
- [94] Fang L, Li H, Wang L, Hu J, Jin T, Wang J, et al. MicroRNA-17-5p promotes chemotherapeutic drug resistance and tumour metastasis of colorectal cancer by repressing PTEN expression. *Oncotarget* 2014;5(10): 2974-87.
- [95] Ghofrani-Shahpar M, Pakravan K, Razmara E, Amooie F, Mahmoudian M, Heshmati M, et al. Cancer-associated fibroblasts drive colorectal cancer cell progression through exosomal miR-20a-5p-mediated targeting of PTEN and stimulating interleukin-6 production. *BMC Cancer* 2024;24(1):400.
- [96] Mogilyansky E, Rigoutsos I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ* 2013;20(12): 1603-14.
- [97] Wei H, Yang Z, Lin B. Overexpression of long non coding RNA CA3-AS1 suppresses proliferation, invasion and promotes apoptosis via miRNA-93/PTEN axis in colorectal cancer. *Gene* 2019;687:9–15.
- [98] Valafar B, Zaravinos A, Bonavida B. Cross talk between the circadian clock proteins and TP53 in cancer and therapeutic significance. *Crit Rev Oncog* 2021; 26(4):19–36.
- [99] Yan H, Xue G, Mei Q, Wang Y, Ding F, Liu M, et al. Repression of the miR-17-92 cluster by p53 has an important function in hypoxia-induced apoptosis. *EMBO J* 2009;28(18): 2719-32.
- [100] Yip H, Papa A. Signaling pathways in cancer: therapeutic targets, combinatorial treatments, and new developments. *Cells* 2021;10(3):659.