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S.Giovanni Rotondo, 18 /11/2013

Editor-in-Chief
World Journal of Gastroenterology

Dear Editor,

please, find enclosed a copy of our REVISED manuscript 5469 entitled “**The circadian clock circuitry in colorectal cancer**” that we are submitting as a review article upon your invitation

All the referee’s concerns have been addressed.

All authors have read and approved the final version of the manuscript, which has not been previously published, nor is being considered for publication elsewhere in whole or in part in any language, except as an abstract.

Looking forward to hearing from you, I remain sincerely yours

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REVIEWER n. 1

In this reviews, authors describe relationship between circadian clock and colorectal cancer. Varieties of recent studies have reported that dysfunction of circadian clock leads to cancer and that abnormal regulation of circadian clock is observed in patients with cancer. To date, there are varieties of review papers dealing with the connection of circadian clock and cancer. However, there is no review paper focusing on the connection between circadian clock and colorectal cancer. Thus, I think that this review will provide important information to researchers from broad fields.

I have several comments on this review, which are summarized below.

Several descriptions regarding the circadian clock are not accurate.

(1) Page2 lanes 25-27

The core circadian clock proteins in mammals are CLOCK, NPAS2, Brain muscle Arnt like 1 and 2 (BMAL1 and BMAL2), CRY1/2, PER 1/2. In mammals, TIME and TIPIN are not critical for circadian regulation. In addition, Per3 KO mouse does not show any circadian phenotype. Many researchers believe that Per3 is one of the output genes, not the core clock gene. Please check the following paper. Zheng B et al. Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. *Cell*. 2001 105, 683-94.

I recommend authors to re-write this sentence.

We thank the referee for the suggestion. We have corrected as suggested

The molecular clock responsible for the generation of circadian rhythmicity consists of a set of interlocking transcription-translation feedback loops that complete one cycle each day and are driven by the core clock genes encoding the circadian proteins BMAL1/2 (ARNTL/2), CLOCK (or its paralog NPAS2), PERIOD (PER) 1-2, CRYPTOCHROME (CRY) 1-2[16]. Other proteins that in some way are related to the clock gene machinery are represented by PER3, TIMELESS, Timeless-Interacting Protein (TIPIN), and protein kinases. The genes PER1-2 and CRY1-2 are transcriptionally activated by the basic helix-loop-helix/PAS (Period, Aryl-hydrocarbon-receptor, Single minded)-transcription factors CLOCK and BMAL1, which heterodimerize and bind to E-box enhancer elements in the promoters of these genes[16]. In turn, PER and CRY proteins form a repression complex that translocates back into the nucleus and interacts with CLOCK and BMAL1, hindering their activity. PER3 is believed to be the product of one of the output genes, more than a core clock gene, considering that Per3 KO mouse does not show any circadian phenotype, whereas PER1 and PER2 play an essential role for the molecular clockwork

(2) Page 2 lane 25

Brain muscle Arnt like (BMAL) or MOP3 is more frequently used in the circadian clock field than ARNTL is. NPAS2 is the paralog of CLOCK. Thus, the description of “CLOCK (NPAS2)” is not correct.

We have corrected as suggested

(3) Page2 lanes 25-27

There are so many kinases involved in circadian regulation, such as GSK-beta and JNK. If authors put CK1-epsilon as the core clock component, they need to put them all, which would take space. I would like to recommend authors to remove CK1-epsilon from the original sentence.

We have corrected as suggested

(4) Page2 lane 46

O-GlcNAcylation has been recently reported to be an important post-translation modification for circadian regulation in mammals (Please see the following references.)

I recommend authors to include this modification in the sentence.

Kaasik K et al. Glucose sensor O-GlcNAcylation coordinates with phosphorylation to regulate circadian clock. *Cell Metab*. 2013 17, 291-302.

Li MD et al. O-GlcNAc signaling entrains the circadian clock by inhibiting BMAL1/CLOCK ubiquitination. *Cell Metab*. 2013 17, 303-10.

We agree with the referee. We have added this issue and references as suggested

In turn, GSK3 β regulates the activity of O-GlcNAc transferase, which works as a metabolic sensor gauging the nutrient flux into the hexosamine biosynthesis pathway, and fine-tuning the regulation of the circadian clock through reversible change of structure and transcriptional activity of the circadian proteins CLOCK and Period, as well as stabilization of BMAL1 and CLOCK as a result of inhibition of their ubiquitination

(5) Page2 lanes 46-48

Casein kinases also have been reported to phosphorylate CRY1 and BMAL1.

GSK3-beta also has been reported to phosphorylate CRY2, PER2 and CLOCK.

I recommend authors to re-write sentence. The following paper summarized post-translational modifications of clock proteins in its table 2, and will be helpful.

Uchida Y et al. A common origin: signaling similarities in the regulation of the circadian clock and DNA damage responses. *Biol Pharm Bull*. 2010, 33, 535-44.

We thank the referee for the precious suggestion. We have rewritten the sentence according to the table2 in the reference suggested

Phosphorylation is operated by protein kinases, such as casein kinase (CK)1- ϵ (encoded by CSNK1E), which targets the proteins BMAL1, PER1, PER2, and CRY1, CK2, which targets BMAL1 and PER2, adenosine monophosphate (AMP) activated kinase (AMPK), which targets the CRY proteins, and glycogen synthase kinase-3 β (GSK3 β), which targets the proteins CLOCK, BMAL1, PER2, CRY2, and in the absence of GSK3 β -mediated phosphorylation BMAL1 becomes stabilized, decreasing the dependent circadian gene expression

(6) Page2 lane 46

Authors mentioned SUMOylation of clock protein (BMAL1). But they did not put reference for the sentence. I recommend them to put following references or the proper review paper.

Cardone L et al. Circadian clock control by SUMOylation of BMAL1.

Science. 2005, 309, 1390-1394.

Lee J et al. Dual modification of BMAL1 by SUMO2/3 and ubiquitin promotes circadian activation of the CLOCK/BMAL1 complex. Mol Cell Biol. 2008, 28, 6056-6065.

We have added the sentence according to the suggestion and the reference suggested

Furthermore, BMAL1 is SUMOylated in vivo on Lys259, CLOCK is necessary to stimulate this post-translational modification, and BMAL1 SUMOylation and activation oscillate with circadian rhythmicity in the mouse liver

(7) Page3 lanes 5-6

In reference 30 (Nakahata Y et al Cell 2008), they did not report that SIRT1 interacts with CLOCK:BMAL1 complex in circadian manner, instead they found that SIRT1 interaction with CLOCK:BMAL1 is not time-dependent and further that the NAD⁺-dependent SIRT1 activity is changed in circadian manner. Circadian regulation of SIRT1 activity has been reported to be dependent on CLOCK:BMAL1-mediated regulation of Nampt 1 expression.

(Please see following papers.)

Nakahata Y et al. Circadian control of the NAD⁺ salvage pathway by CLOCK-SIRT1.

Science. 2009, 324, 654-657.

Ramsey KM et al. Circadian clock feedback cycle through NAMPT-mediated NAD⁺ biosynthesis.

Science. 2009, 324, 651-654.

In addition, Nakahata et al have reported that SIRT1 deacetylates BMAL1 and they did not report the SIRT1-mediated deacetylation of PER2. The SIRT1-dependent BMAL1 acetylation in vivo has been also reported from the other group. (Please check the following paper.)

Chang HC et al. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell. 2013, 153, 1448-60.

I recommend authors to rewrite the sentence.

We have rewritten the sentence according to the referee's suggestions and added the correct references

Acetylation is operated by CLOCK, whereas deacetylation is operated by SIRT1, a type III NAD⁺-dependent histone/protein deacetylase that is required for high-magnitude circadian transcription of several proteins encoded by core clock genes, including BMAL1, PER2, and CRY1, counterbalances the histone acetyltransferase activity of CLOCK, and promotes the deacetylation and degradation of PER2. The interaction of SIRT1 with CLOCK:BMAL1 is not time-dependent, whereas the NAD⁺-dependent SIRT1 activity changes in circadian manner, and the circadian regulation of SIRT1 activity depends on CLOCK:BMAL1 mediated regulation of expression of nicotinamide phosphoribosyltransferase (NAMPT), the rate limiting enzyme involved in NAD⁺ synthesis

(8) Page 5 lane 7

CK1 epsilon would not be the core clock component. Thus, the sentence needs to be rewritten.

We have corrected as suggested

(9) Page 7 lanes 26-28

The description of “ ARNTL (BMAL1) protein represses c-Myc” is correct. However, the reference for this sentence should not be the ref # 109 (Gorbacheva VY et al. PNAS 2005), and it should be the ref # 49 (Fu L et al. Cell 2002)

We have corrected the reference as suggested

(10) I would like to recommend authors to put the table and/or figure, which summarize the connection between circadian gene mutations (or circadian dysfunctions) and colorectal cancer. In the part of “ The biological clock in colorectal carcinogenesis”, authors described valuable information. I believe that the table and/or figure would be helpful for the reader to understand what authors described.

We have added an explanatory figure-scheme

I also would like to recommend authors to subdivide the the part of “ The biological clock in colorectal carcinogenesis”. I think that this part is too long.

We have subdivided this section as suggested by the referee adding the sub-heading “The clock genes in human colorectal cancer”

(11) There are many English or spelling mistakes. I would like to ask authors to check their manuscript more carefully. I described several examples of mistakes.

Page 1, lane 3 from the last; “CRC onset and progression is” should be “CRC onset and progression are”.

Page 2, lane 18; “The central pacemaker and master oscillator is” should be “The central pacemaker is”.

Page 2, lane 6; In this part authors described “beta-Catenin”. In other part (ex. Page 3 lane 6 from the last), they described “beta-catenin.

Same problem for “cryptochrome (or Cryptochrome)” (See page 6, lanes 38-43)

Page 4, lane 2; “C-Myc” should be “c-Myc”.

Page 6, lane 13; “detoxification shows” should be “detoxification show”.

Page 6, lane 44; “been found correlated” should be “been found to be correlated”.

We have corrected these errors and checked the manuscript

(11) There are problems of sequence of tense through the manuscript.

We have addressed this issue checking for continuity

REVIEWER n. 2

Reviewed by 02521098

Manuscript Number 5469

Manuscript Title The circadian clock circuitry in colorectal cancer

Review Time 2013-10-04 22:16

Comments To Authors: Interesting topic. however, 2 issues should be paid attention to:

1. The epidemiological data is too old, mostly in 2005, and I suggest updating epidemiological data.

We thank the referee for the suggestion, we have updated epidemiological data

2. References is nonething new, basically in 2005, also should be updated.

We agree with the referee and we have updated the references, expecially of reviews, and we have maintained some of the ground-breaking scientific articles

Dear Dr. Mazzocchi,

Thank you very much for accepting our invitation to contribute high-quality manuscripts to the World Journal of Gastroenterology. The columns in which your submitted manuscript can be published are as follows:

Review, entitled: The circadian clock circuitry in colorectal cancer

The format requirements for manuscripts submitted to World Journal of Gastroenterology can be found at <http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15>.

There is no restriction on number of words, figures, tables and references. Your paper will be published free of charge after peer review.

Please submit your manuscript online via the Express Submission and Peer-review System (<http://www.wjgnet.com/esps/NavigationInfo.aspx?id=13>).

Please choose the column in the above list and indicate your ID (02494580) in the cover letter. The closing date for submission is 2013-09-28.

Thank you for your attention,

Best regards,

Lian-Sheng Ma, President and Company Editor-in-Chief

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