



## Commentary

## Deregulation of the circadian clock machinery: A novel biomarker for anti-angiogenic drug resistance in colorectal cancer

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Cancer statistics is alarming; latest reports estimated 18.1 million of new cases and 9.6 million of deaths from cancer worldwide [1]. Colorectal cancer (CRC) is among the most commonly diagnosed cancers (6.1% for incidence), and a leading cause of cancer mortality (9.2%) [1]. Therefore, a great effort is needed to fight against this pandemic. One of the biggest issues in cancer patients, considering disease-free survival and quality of life, is the favorable or unfavorable response to drug treatment. The therapy response often depends by drug resistance, recognized as the different mechanisms adapted by cancerous cells to resist and eventually overcome treatment [2]. Understanding these mechanisms, which include drug transport and metabolism, mutation and amplification of drug targets, or presence of genetic variants that may differently predispose to drug response [2], is crucial to elude this major problem in oncology and to identify novel anti-cancer strategies.

When metastatic CRC (mCRC) is diagnosed, the expectancy of survival is around 10%, and is strongly dependent by the response to therapy. Inhibitors of vascular endothelial growth factor A (VEGFA), such as bevacizumab (Beva), are now routinely incorporated into the first line treatment of mCRC patients [3] with the final goal to inhibit neoangiogenesis, a critical step in carcinogenesis. Clinical trials demonstrated that addition of Beva to chemotherapy improved overall survival (OS) by 12 months compared with placebo, and recent results are even more promising [3]. However, an increasing number of mCRC patients present intrinsic refractoriness and acquired resistance to Beva [4]. Although several mechanisms have been explored and hypothesized to explain the reaction to the drug [4], many questions remain unresolved.

Recently, the circadian clock has been shown to play a pivotal role in cancer by regulating cell proliferation, metabolism, inflammation and response to DNA damage [5]. Alterations in sleep/activity rhythms accelerate cancer growth, and a significant connection between variations in the circadian clock and cancer therapy response has been suggested [5]. Intriguingly, the circadian clock also regulates angiogenesis [6]. Specifically, the transcription factor Aryl Hydrocarbon Receptor Nuclear Translocator-Like (ARNTL/BMAL1) coordinates the circadian rhythm of

VEGFA expression [6]. Therefore, based on these data, Burgermeister et al. published a fascinating study in EBioMedicine [7] that aimed to evaluate whether the circadian clock machinery is implicated in resistance to Beva in CRC. The authors conducted a sophisticated translational research study reporting results from *in vitro* to *in vivo* and patients' analyses. The beneficial role of Beva was confirmed in terms of its ability to reduce CRC incidence and multiplicity using two different mouse models of CRC (GEMM and HCT116 xenografts) [7]. However, the failure of Beva to reduce microvessel density (MVD) in these animals, evaluated by CD31 staining positivity, raised new questions on the mechanisms of action and resistance pathways not directly correlated to neoangiogenesis inhibition by Beva.

Furthering evidence showing that Beva was unable to abolish the high expression levels of BMAL1 and VEGFA in the intestinal organs of animals with CRC suggested, as an innovative discovery, a potential role of the circadian clock in resistance to Beva. Although mice treated with a combination of FOLFOX (chemotherapy agent) and Beva developed smaller tumours compared with those receiving single compounds or vehicle, Beva not only failed to reduce BMAL1 positivity in the tumour tissue but rather increased its expression. Consistent with these *in vivo* findings, Burgermeister et al. [7] employed a mechanistic approach in human CRC cell lines to demonstrate that REVERBA, a transcription factor activated by BMAL1, bound *Retinoic Acid Receptor-Related Orphan Receptor Alpha responsive element* (RORE) in the human VEGFA promoter, thereby increasing VEGFA synthesis. Interestingly, REVERBA and BMAL1 mutually amplified their activities at the VEGFA promoter, and Beva was not able to blunt this effect, whereas inhibition of REVERBA significantly diminished tumour cell proliferation. This discovery is of particular importance, especially for clinical oncologists, since it may open novel therapeutic strategies for the treatment of mCRC. Based on the present findings, REVERBA antagonists, such as SR8278, which has been previously developed to explore circadian and metabolic functions [8], should be tested in clinical trials to counteract resistance to Beva in mCRC patients. Indeed, data on SR9009 and SR9011, two specific REVERBA agonists, which have been shown to be lethal to cancer cells and oncogene-induced senescent cells [9], suggest particular utility in this setting. Other cellular escape mechanisms implicated in resistance to Beva during tumour progression and their relationship with the circadian clock should be considered to definitively

DOI of original article: <https://doi.org/10.1016/j.ebiom.2019.07.004>.E-mail address: [ddellamorte@med.miami.edu](mailto:ddellamorte@med.miami.edu).<https://doi.org/10.1016/j.ebiom.2019.08.003>2352-3964/© 2019 The Author. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

define the drug target pathways causative for resistance to Beva. These mechanisms may include upregulation of alternative angiogenic factors, increased recruitment of neutrophils, autophagy, and acquiring dormant and quiescent states of tumours, among others.

In this respect, the commented study [7] moved forward demonstrating that BMAL1 was reduced by ~50% in tumours of mCRC patients compared with the normal colon tissue, and low levels of BMLA1 were present in patients with a good response to Beva therapy and stable disease. Conversely, high BMAL1 expression was associated with reduced progression-free survival (PFS) and poor clinical outcome following Beva treatment. Therefore, BMLA1 is proposed as a potential predictive biomarker for Beva resistance in mCRC patients, notwithstanding that larger confirmatory clinical studies are required. In the genetic analysis, single nucleotide polymorphism (SNP) rs11022780 in the BMAL1 (ARNTL) gene was shown to have a significant association with OS, while rs2279287, rs7938307 and rs7396943 SNPs were correlated with shorter PFS. Since the analysis was well adjusted for demographic and clinical confounders, these data suggest that different functions of the BMLA1 protein, based on different transcription levels, may have a differential impact on CRC progression and clinical performance. These data are in agreement with a report on Bmal1 knockdown which demonstrated opposite carcinogenic effects of this gene [10]. However, for the genetic study, samples were obtained from the III TRIBE trial cohort composed of patients recruited from 34 Italian oncology units, therefore mainly from Caucasian patients. A more robust genetic validation analysis of these data in other ethnicity cohorts with higher incidence of CRC, such as in the USA, is required. Studies with knockout animal models for molecules implicated in circadian clock pathways along with studies using inoculation of target SNPs in transgenic mice, would be also very useful.

Burgermeister et al. [7] have convincingly highlighted the role of the circadian clock in cancer and suggested a novel therapeutic strategy

to counteract resistance to Beva in mCRC patients. Research engaged in the development of anti-cancer drugs or identification of mechanisms of drug-resistance in mCRC and other tumours should now consider circadian rhythm variability. The mortality for mCRC is unfortunately still too high, therefore such studies, as the discussed one [7], are warranted in the future.

## Disclosure

I have nothing to disclose.

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