Editorial

Cancer prevention through better sleeping habits: the circadian clock and hepatocellular cancer

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The incidence of hepatocellular cancer (HCC) has been steadily increasing in the United States for the last several decades, and currently HCC constitutes the fastest growing cause of cancer deaths in the country. The majority of HCC occurs in the setting of chronic liver disease with viral infections [hepatitis B virus (HBV) or hepatitis C virus (HCV)] and alcohol-related liver disease being the most common risk factors. Recently non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) have been recognized as conditions predisposing to the development of end stage liver disease and HCC (1). It is estimated that in the next 10 years, NASH will become the leading indication for liver transplantation in the Western world (1,2).

NAFLD is extremely common in the Western population, and according to some reports roughly 30% of population is affected by it to a certain degree. The development of steatosis is a component of a complex, multifactorial process that not only involves the liver, but also effects a range of metabolic processes in all body systems including certain epigenetic processes and the circadian rhythm (3). This process, commonly referred to as the metabolic syndrome, usually manifests with the initial development of insulin resistance and liver steatosis. Subsequent oxidative stress and chronic inflammation in the liver lead to the development of hepatic steatosis, followed by fibrosis, and finally cirrhosis. Although liver cirrhosis is a powerful driver for the development of HCC, it is not uncommon for carcinoma to develop in the setting of NAFLD or NASH before progressing to cirrhosis (2,3). A recent publication by Kettner and colleagues proposes that dysregulation of the circadian clock, also termed chronic jet lag, can independently lead to the development of liver cancer in a stepwise process beginning with the development of fatty liver disease then progressing through steatohepatitis, fibrosis, and eventually developing into HCC (4).

The biological clock is not unique to mammals as it plays a role in the homeostasis of all eukaryotes (5,6). It is the circadian clock, not outside environments that determines behavior patterns and oscillations in the physiology of species from single cell organisms to mammals (7). In humans, the circadian rhythm is governed by the suprachiasmatic nucleus (SCN), which acts as the "central clock", and coordinates circadian rhythmicity of multiple organ systems. The most potent exogenous stimulus that influences circadian pattern is ambient light. Most, if not all organs, have their own biological clocks, which act as self-sustained autonomous oscillators that are connected by positive and negative feedback loops to the SCN by means of neuroendocrine and autonomic nervous systems. Many processes such as immune function, hormone production, secretions of the gastrointestinal tract, body temperature, sleep/wake cycles, as well as gene and protein expression, are regulated by a number of clock genes and proteins, and the expression of these genes varies between different peripheral tissues in the body (6).

The major players in the regulation of the circadian

clock at the molecular level are the transcription factors CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1), the genes *hPer1*, *-2*, and *-3* (human period), and *Cry1* and *-2* (cryptochrome) and their respective protein products PER and CRY. CLOCK-BMAL1 heterodimer controls circadian oscillations by upregulating PER and CRY proteins, which in turns through negative feedback loop repress activity of the CLOCK-BMAL1 complex. PER and CRY are ultimately degraded, allowing the process to proceed in a cyclical fashion; a process that usually takes about 24 h. Products of several metabolic processes ongoing throughout a person's body influence the levels of transcription factors and genes regulating the biological clock, and may ultimately alter circadian rhythm (5,6).

Early in evolution and currently in non-developed regions of the world, humans are awake during the day and are asleep at night. However, in modern industrialized societies, people's circadian rhythm is often altered by artificially lit environments, shift work and transcontinental travel. All these factors contribute to interruption of circadian hemostasis, which has been implicated in the development of multiple diseases, ranging from sleep and mood disorders to hypertension, the metabolic syndrome, and cancer (5-7). Cell cycle dysregulation is a cardinal feature of cancer cells and the cell cycle is strongly linked to a person's biological clock. Many common, oncogenic transformations lead to downstream mutations of circadian genes and proteins that in turn further contribute to tumorigenesis by affecting tumor suppressors and proto-oncogenes that naturally display circadian rhythmicity. Multiple cell cycle regulatory genes have been associated with circadian clock including c-Myc, p53, cyclin D, and RAS family (5,8,9). Circadian genes have been implicated in multiple cancers including hematologic malignancies, squamous cell carcinoma of head and neck, breast and gastrointestinal caners (5-9). Although the exact mechanism is not clearly understood, it is hypothesized that alterations in circadian rhythm result in epigenetic modifications and chromatin remodeling further contributing to carcinogenesis (6,8).

Some known circadian genes, in particular PER, CRY and BMAL1 were found to be downregulated in patients with squamous cell carcinoma of head and neck. These alterations were associated with larger tumor size, deeper invasion, more advanced cancer stage and shorter survival (10). In gastric cancer PER2 was more likely to be upregulated in comparison to normal stomach tissue, and expression of CRY1 was positively correlated with more advanced tumor stage (11). Acute lymphocytic leukemia and non-Hodgkin lymphoma are also characterized by circadian deregulation of the c-Myc protein resulting from hyper-methylation of the BMAL1 gene (12). Downregulation of clock genes has also been reported in gynecological malignancies of endometrial (13) and ovarian (14) origin.

Breast cancer is one of the best studied malignancies with respect to the role of circadian rhythm in the process of tumorigenesis. Many have reported that the CLOCK gene has significantly higher expression in mutated breast cancer tissue in comparison to unaffected tissue. Moreover, estrogen-negative tumors, which carry worse prognosis, upregulate CLOCK gene in comparison to hormone positive tumors (15). A reverse relationship is also observed for PER genes, with decreased expression conferring higher risk of breast malignancy and worse prognosis (6). Interestingly, there are several publications which focus mostly on nurses and airline crews that report increased incidence of breast cancer among women working night shifts, with relative risk of 1.2–1.8 (16-18). However, these studies focused on nocturnal exposure to light and decreased levels of melatonin as risk factors, not on the molecular mechanism involved in circadian rhythmicity. That being said, the epidemiologic data demonstrating an association between nightshift work and increased risk of breast malignancy is intriguing and warrant more in depth studies.

There is a breadth of information derived from studies of HCC in animal that demonstrates a strong correlation between altered biological clock genes and malignant transformation of the liver. Filipski *et al*. compared two cohorts of mice exposed to known liver carcinogen diethylnitrosamine (DEN) with one group being raised in normal 12-hour day-night conditions, and the other group was submitted to chronic jet lag. The mice from the second group were found to have an increase number and larger liver tumors than the controls (19). In addition, several studies looking at HCC tumor samples have also shown a correlation between an altered biologic clock and the development of HCC (20-22). To our knowledge, there is no published epidemiologic data that explores the incidence of HCC in subjects working unusual hours, especially night shifts.

In their recent project, Kettner *et al*., using a mouse model, showed that dysregulation of circadian genes and the biological clock leads to activation of several oncogenic

pathways (4). In normal, non-jet-lagged mice, cholestasis is prevented by farnesoid X receptor (FXR), which also acts as an inhibitor of several inflammatory genes and steatosis. When the circadian clock is dysregulated, the normal endocrine negative-feedback loop is disturbed leading to liver damage via the development of cholestasis. In a stepwise process, this then lead to NASH and eventually HCC. Furthermore, cholestasis causes activation of the constitutive androstane receptor (CAR), a key factor in the metabolism of exogenous substances and a known factor in mutagen-induced liver cancer in mice. The study showed that not only dysregulation of circadian rhythm leads to the alterations of the key genes in biological clock, but that it is also capable of initiating the process of carcinogenesis independently from mutation of circadian genes.

The fact that liver cancer is closely tied to the inner biological clock, opens the door to the development of new treatment strategies. Chronotherapy is based on the concept that delivering of chemotherapeutic agents at certain times of the day coordinated with circadian rhythm, and thus the cell cycle, may not only decrease toxicity to the patients but increase the efficacy of treatment (15,23). The antimetabolite chemotherapeutic agents that are most effective against actively dividing cells were first to be shown to vary in their efficacy depending on the time of administration. In the study of children with acute lymphoblastic leukemia (ALL), the patients who received their chemotherapy in the evening had better survival than those who were treated in the early morning hours (24). Patients with colorectal cancer were also found to have a higher response rate when treated with chronomodulated FOLFOX infusions, as compared to patients who received daily boluses of the chemotherapy (25). In addition, it has been shown in mice studies that metabolism of platinum based agents is most robust in the middle of day when patients are active versus 5-fluorouracil (5-FU) which is metabolized faster during rest hours. Clinical studies confirmed these pharmacokinetic studies and demonstrated that infusion of 5-FU and oxaliplatin at times synchronized with a person's biological clock resulted in less adverse events in comparison to when they were administered at random times during the day (26).

A better understanding of how the biological clock is intertwined in metabolic processes and carcinogenesis will hopefully yield attractive targets not only for treatment but also for prevention of liver disease and HCC. Eliminating derangements of circadian rhythm and maintaining homeostasis of bile acids may help prevent the development of NAFLD and NASH. Although more clinical studies are needed, the future of cancer prevention and better liver health may be provided simply by a doctor's prescription for a good night sleep.

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