Biological Clock in Liver Cancer

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The biological clock controls at the molecular level several aspects of mammalian physiology, by regulating daily oscillations of crucial biological processes such as nutrient metabolism in the liver. Disruption of the circadian clock circuitry has recently been identified as an independent risk factor for cancer and classified as a potential group 2A carcinogen to humans. Hepatocellular carcinoma (HCC) is the prevailing histological type of primary liver cancer, one of the most important causes of cancer-related death worldwide. HCC onset and progression is related to B and C viral hepatitis, alcoholic and especially non-alcoholic fatty liver disease (NAFLD)-related milieu of fibrosis, cirrhosis, and chronic inflammation.

Keywords: hepatocellular carcinoma (HCC) ; circadian clock ; chronotherapy

1. Introduction

Worldwide, liver cancer ranks second among the principal causes of cancer-related death and fifth in men and ninth in women among the most commonly diagnosed cancers, respectively, with more than 800,000 new cases in 2018 and hepatocellular cancer (HCC) accounting for 70–85% of all liver cancers [1][2]. Recent availability of effective direct antiviral agents targeting hepatitis C virus (HCV) NS3/4A (protease), NS5B (polymerase), and NS5A (nonstructural) protein has greatly curtailed the causative role of HCV infection in hepatocarcinogenesis and the most important risk factor for liver cancer is actually represented by excess body fat. The global epidemics of obesity, metabolic syndrome, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD) facilitated the quick rise in HCC prevalence [3]. NAFLD may progress to nonalcoholic steato-hepatitis (NASH), hallmarked by steatosis with necroinflammation, and in due course to fibrosis, cirrhosis, and HCC. For HCC developing without cirrhosis, associated factors may include inflammatory, metabolic, endocrine, bile acid flux, and gut microbiome derangements associated with obesity and liver fat accumulation [3].

2. The Circadian Clock Circuitry and the Molecular Mechanisms of Hepatocellular Carcinogenesis

Hepatic carcinogenesis is linked to the ongoing failure of mechanisms managing basic cellular processes, such as cell cycle, proliferation, differentiation, apoptosis, DNA damage response, autophagy, xenobiotic detoxification, anabolic/catabolic processes, and oxidation-reduction reactions with reactive oxygen species production/antioxidant defenses balance [4]. Key embryogenesis-related and oncogenic pathways have been recognized by genetic studies as deranged as well, among which WNT/β-catenin, proliferation, and hepatoblastoma-like pathways, at present are not easily druggable for targeted cancer therapy [4]. The greater part of the aforementioned biological processes and signaling pathways are hallmarked by rhythmic activity fluctuations with about 24-h (circadian) periodicity [5][6][7].

The nycthemeral rhythmicity featuring behavior (locomotor activity, eating/fasting, sleeping/waking) and physiology (temperature, blood pressure, hormone secretion) of living beings is controlled by the circadian timing system. This time-keeping system is organized as a hierarchical network comprising self-sufficient biological oscillators in the hypothalamic suprachiasmatic nuclei (SCN) and in peripheral tissues. The circadian timing system operates by transduction and integration of photic signaling (outdoor light levels/indoor lighting conditions) and grants organism/species survival advantage through appropriate anticipation of expected environmental changes. The central pacemaker (SCN) entrains peripheral tissues oscillators by means of cues, such as hormone (cortisol, melatonin), physical (temperature fluctuations), and neural (autonomic nervous system fibers) outputs. This complex and rhythmic signaling drives behavioral cycles (food craving and intake, rest-activity), nervous, cardio-vascular, gastro-intestinal, and musculoskeletal system function in synchrony with metabolic pathway activation, transcriptome-metabolome oscillations, oxidation-reduction reactions, and nutrient level fluctuations [5][6][7].

At the cellular level 24-h rhythms are generated by a molecular mechanism maneuvering transcription-translation feedback loops hard-wired by intertwining circadian genes and proteins, precisely ARNTL/2 (BMAL1/2), CLOCK (or its

paralog NPAS2), PERIOD (PER) 1–3, CRYPTOCHROME (CRY) 1–2, REV-ERBs/RORs nuclear receptors and TIMELESS. The transcription factors CLOCK and BMAL1 heterodimerize, bind to E-box enhancer elements in the promoters of the genes *PER1-3* and *CRY1-2* and turn on their transcription, whereas PER and CRY protein complexes impede their transcriptional activity [8]. TIMELESS intermingles with TIPIN and deals with DNA replication and damage response, above all S-phase arrest and signaling pathways mediated by ATR-Chk1 and ATM-Chk2 [9]. The nuclear receptors REV-ERB α/β (encoded by *NR1D1*/*NR1D2*) and ROR α/γ control *ARNTL* rhythmic transcription competing at ROR-responsive elements (RORE) of its promoter [10][11][12]. Post-translational modifications of circadian proteins, represented by phosphorylation, SUMOylation, acetylation and deacetylation, O-GlcNAcylation [13], hold up proper functioning of the molecular clockwork. In particular, phosphorylation is operated by several protein kinases, such as casein kinase (CK)1-ε (encoded by *CSNK1E*), adenosine monophosphate (AMP) activated kinase (AMPK), and glycogen synthase kinase (GSK)-3β [13][14]. In addition, ARNTL is SUMOylated with circadian rhythmicity in the mouse liver [15][16]. Acetylation is managed by histone/protein acetyl-transferases, such as CLOCK, while deacetylation is activated by histone/protein deacetylases, such as the NAD+-dependent SIRT1 [17][18][19][20]. The molecular clockwork drives the rhythmic transcription of clock controlled genes, such as the PAR bZIP transcription factors DBP, TEF, HLF, which in turn drive the expression of thousands of genes, among which are cell cycle progression regulators (Cyclin D1, Cyclin A, Mdm-2, c-Myc, WEE-1, GADD45A) and tumor suppressor genes/oncogenes as well [21][22][23][24][25]. In pre-neoplastic liver lesions of Fischer 344 rats with induced carcinogenesis through the resistant hepatocyte model and in c-Myc/TGF-α transgenic mice, up-regulation of c-Myc, cyclin D1, cyclin A, and E2F1, involved in cyclin D1-CDK4, E2F1-DP1 complexes and pRb hyper-phosphorilation was identified [26][27]. Changes of G1 to S cell cycle phase transition ensuing from these derangements take part in human HCC, as well [28]. A proper functioning of the biological clock demonstrates tumor suppressing potential, whereas circadian rhythmicity disturbance, like that provoked by shift work in humans and chronic jet lag (CJL) in animal models, is an independent risk factor for HCC: for instance, it worked as tumor promoter in mice exposed to the hepatic carcinogen diethylnitrosamine (DEN) in combination with 8-h advance of light onset every 2 days [29]. Accordingly, chronic circadian disruption and ablation of Steroid Receptor Coactivator-2 (SRC-2), a crucial metabolic transcriptional co-regulator in SCN and liver, altered behavioral activities and metabolic homeostasis in SRC-2(-/-) mice, leading to NAFLD, NASH, and HCC [30]. Furthermore, animal experiments performed in wild type and core clock genes mutated mice revealed an evident augment of early NAFLD onset with progression to NASH, fibrosis, and, in due course, HCC. The exploration of the molecular mechanisms revealed deregulation of liver metabolic genes enriching nuclear receptor-controlled cholesterol/bile acid and xenobiotic metabolism pathways and suggested a protective role for farnesoid X receptor (FXR) and a pro-tumorigenic role for constitutive androstane receptor (CAR) [31]. Interestingly, evaluation of circadian genes and protein expression in human HCC and matched non-tumor specimens showed reduced expression levels of PER1, PER2, PER3, CRY2 in HCC, with over-expression of the histone methyltransferase Enhancer of zeste homolog 2 (EZH2) and promoter methylation, but no genetic mutations [32][33], and experiments performed in vitro challenging the PLC/PRF/5 human HCC cell line with CoCl2 for 24 h at increasing concentration (50, 100, and 200 μM) to mimic an hypoxic environment showed an additional effect of hypoxia in circadian genes' deregulation in expression of in HCC [34].

3. The Biological Clock and Systemic Therapy in Hepatocellular Carcinoma

The role of the biological clock in pharmaceutical intervention for the treatment of NAFLD and the effects of dietary habits changes, especially in relationship to time-of-day of food consumption, in the organism's metabolic homeostasis and in the physiopathology of hepatic steatosis have been extensively and comprehensively described in a recent review [35], so here we will limit ourselves to mentioning some fundamental concepts regarding a potential role of meal timing and frequency scheduling in NAFLD-NASH-HCC progression. In the last two decades, numerous scientific studies have tried to clarify the role of the biological clock in the regulation of metabolic pathways, while little is known about the effects of metabolism intermediates on the functioning of the biological clock, especially in conditions of alterations of the metabolic homeostasis. For example, the restriction of nutritional intake to specific time windows, i.e., time restricted feeding (TRF), has direct effects on behavioral and physiological patterns and determines a response of the organism apt to anticipate the moment of possible food intake, a phenomenon called food anticipatory activity, whose regulatory centers and bio-molecular mechanisms are still poorly defined [36]. The effects of the temporal restriction of food intake are better known, especially in rodents when used as experimental models. Usually the fasting/feeding cycle corresponds temporally to the sleep/wake cycle, differing in the various animal species, which can be diurnal (active by day), nocturnal (active by night), or crepuscular (active especially during twilight, for example at dawn and sunset). When the availability of food is temporarily restricted and out of phase with the body's natural cycles, the expression of circadian genes is also decoupled in the peripheral tissues with respect to the SCN, which will continue to be synchronized to the natural light/dark cycle. Furthermore, TRF was shown to have more favorable effects on physiological (weight, visceral fat), metabolic (glycemic

levels, glucose tolerance), and hormonal (serum insulin and leptin levels) parameters with respect to ad libitum diet, even when the caloric intake was represented from a diet high in fat [37]. Another option is represented by intermittent/periodic fasting and fasting-mimicking diets, capable to bring on visceral fat decrease with no lean body mass change, hasten immune system renewal, hinder and partly undo bone mineral density loss, halt and reduce incidence of inflammatory diseases and cancer [38]. These data suggest the importance of the temporal characteristics of food intake as well as food abstinence or reduction in order to maintain an adequate energy balance, deriving from the equilibrium between energy intake and expenditure, which in turn corresponds to the sum of basal metabolism, energy expenditure for physical activity, and diet-induced thermogenesis [37][39][40][41][42][43][44]. As far as humans are concerned, the possibility of using time scheduled food intake, i.e., chrono-nutrition, as a therapeutic strategy in conditions of altered metabolism, such as obesity, metabolic syndrome, diabetes mellitus, liver steatosis, as well as NAFLD/NASH-related HCC is a recent proposal, even if knowledge on how timed feeding influences the circadian timing system and the molecular clockwork is still limited. Anyway, recent studies already suggest that the timing of nutrition could translate into a beneficial approach to improve weight loss and metabolic homeostasis in humans and support the possibility to combine timed feeding/fasting-associated interventions with standard therapeutic strategies for neoplastic diseases, liver cancer included, although the physiological mechanisms and molecular signaling pathways implicated in these favorable modifications need to be better defined [45].

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