

# A METABOLIC OSCILLATOR CONTROLS TEMPORAL CHANGES IN LIPID METABOLISM AND REDOX STATUS IN TUMOR CELLS. IMPLICATIONS IN CHEMOTHERAPY SUSCEPTIBILITY

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Introduction: Biological clocks even present in immortalized cell lines, regulate physiological processes in a time-dependent manner, driving transcriptional and metabolic rhythms. The disruption of circadian rhythmicity in modern life (shiftwork, jetlag, etc.) may promote higher cancer risk and metabolic disorders. We have previously demonstrated that glycerophospholipid (GPL) synthesis is highly regulated by the biological clock under physiological conditions in retinal cells (Guido et al 2001, Garbarino-Pico et al 2004, 2005), in the mice liver (Gorne et al 2014), and in fibroblast (NIH3T3 cells) cultures (Marquez et al 2004, Acosta et al 2013). Significant temporal changes were observed in GPL labeling in different cells studied as well as in GPL synthesizing enzyme expression and activity while rhythms were lost after the clock protein PER1 knocked down. The molecular clock drives the expression of activators (BMAL1), repressors (PER1, 2, REV-ERB) and clock-controlled genes (Choline Kinase, etc.) along the 24 h. Nevertheless, little is known about the biological clock function in tumor cells and how its pharmacological modulation could offer selective anticancer strategies. REV-ERBs are circadian repressor components involved in tumorigenesis (metabolism, proliferation and inflammation). SR9009 is a synthetic REV-ERB specific agonist exhibiting potent *in vivo* activity on metabolism and cell viability. Objectives: Here we investigated metabolic rhythms in two human tumor cell lines: the glioblastoma T98G and hepatocarcinoma HepG2 cells. Also, we evaluated whether cells displayed differential time responses to chemotherapy after treatment with bortezomib (BOR) or SR9009. Methods: T98G and HepG2 cells grown in 10% FBS-DMEM were synchronized with 100 nM Dexamethasone (DEX) for 20 min and collected at different times. Cell viability was analyzed by MTT assay at 570 nm after SR9009 (20  $\mu$ M) or BOR (500 nM). Reactive oxygen species levels (ROS) were determined with 2,7-dichlorodihydrofluorescein diacetate (2  $\mu$ M). Lipid droplets (LDs) were stained with Nile Red (1.5  $\mu$ g/ml) and visualized by confocal microscopy and flow

cytometry. Results: In synchronized T98G cells, rhythms of redox state and GPL metabolisms were observed while cell viability significantly changed over time after BOR or SR9009 treatment. Moreover, the temporal changes in susceptibility and the redox cycles were further altered after *Bmal1* knock-down, indicating a cross-talk between the molecular clock and the metabolic oscillators. SR9009 treated cells exhibited significant anti proliferative effects and displayed differential time responses to the treatment. SR9009 treatment decreased ROS levels while BOR increased them. However, both treatments significantly increased LD levels whereas the combined treatment significantly decreased cell viability. Synchronized HepG2 cells displayed significant circadian rhythms in the expression of *Bmal1*, *PER1*, *Rev-Erb* and *ChoK*-like proteins, in the content of endogenous GPLs (PC and PE) and in LD levels (number and size). Remarkably, when the circadian clock was perturbed by knocking down *Bmal1*, LD levels were severely affected and rhythms damped out. Conclusions: Results suggest that an intrinsic metabolic clock continues to function in diverse proliferating tumor cells and the pharmacological modulation of their intrinsic clock severely affected tumor cell metabolism and promote anti proliferative effects.