Circadian control of P-glycoprotein in Hepa1-6 hepatocarcinoma tumor cells and Hepa1-6 tumor-bearing mice

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Circadian clock largely modifies efficacy and toxicity of many anticancer agents. An efflux transporter P-glycoprotein (encoded by *abcb1a/abcb1b* genes in rodents) is a well-known mechanism causing multidrug resistance in cancer patients by extruding anticancer drugs out of tumor cells and its expression is under the control of circadian clock. We aimed to investigate the circadian clock control of P-glycoprotein in Hepa1-6 hepatocarcinoma tumor cells in vitro and Hepa1-6 tumor-bearing mice in vivo. Bioluminescence oscillatory profile reflecting core clock gene *Per2* expression was determined by Real-Time gene expression monitoring device Lumicycle32 in vitro in synchronized mouse *Per2:Luc* Hepa1-6 hepatocarcinoma cells obtained following stable introduction of luciferase reporter gene under the control of *Per2* promoter in Hepa1-6. *Abcb1a/abcb1b* expression rhythms were estimated using qRT-PCR in dexamethasone-synchronized Hepa1-6 cells at 40% and 100% confluence sampled every 3h during 48h. Hepa1-6 tumor cells were subcutaneously injected into each flank of C57BL/6J male mice. 11 days after injection, tumors were palpable (early stage) and reached a size of 2000 mm³ (late stage) after 23 days. Tumor tissues were removed every 3h during 24h from mice at early and late stage. *Per2* and *abcb1a/abcb1b* mRNA expression rhythms were investigated with qRT-PCR in both early and late stage of proliferation. Data were analyzed with Cosinor for rhythm detection. Real-time bioluminescence pattern of *Per2* revealed circadian rhythm in proliferative (Period=24h; $p<0.001$) and confluent states (Period=25h; $p<0.001$) in *Per2:Luc* Hepa1-6 cells. *Abcb1a* and *abcb1b* were expressed rhythmically along 48h in *Per2:Luc* Hepa1-6 cells at 40% confluence (Period=24h; $p=0.01$ and $p<0.001$) but not at 100% confluence (Period=25h; $p=0.70$ and $p=0.76$). *Per2* expression was not rhythmic in hepatocarcinoma cells of tumor-bearing mice in both early ($p=0.08$) and late-stage of tumor progression ($p=0.36$). *Abcb1a* and *abcb1b* were expressed rhythmically along 24h in Hepa1-6 tumor bearing mice in early-stage of tumor progression (Period=24h; $p=0.03$ and $p=0.02$, respectively) but no rhythm was observed in the late-stage of tumor for *abcb1a* and *abcb1b*. We provided stage-specific expression profiles of core-clock gene *Per2* and P-glycoprotein genes *abcb1a/abcb1b*, suggesting that disturbed circadian clock gene expression and disruption of circadian rhythms correlate with tumor development and progression.