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THE LOSS OF THE CIRCADIAN CLOCK GENE *BMAL1* INCREASES TUMOUR INITIATION IN APC^{MIN} MICE

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Background: Circadian rhythms are autonomously running 24h cycles in bodily processes. In animals these rhythms are driven by a molecular time keeper known as the circadian clock. The clock is a transcription-translation feedback loop composed of the transcription factors Bmal1 and Clock as well as their repressors Per and Cry. The circadian clock regulates over 40% of the genome rhythmically. Chronic circadian disruption, in the case of shift work, can lead to pathologies including cancer. Colorectal cancer is most frequently initiated through a mutation in the Wnt pathway regulator, *Apc*. Several studies have attempted to provide a mechanistic link between cancer and circadian clock disruption but the use of mice on mixed genetic backgrounds and poor circadian models have made this link unclear.
Aims: We aim to determine if the circadian clock plays a role in intestinal tumourigenesis.
Methods: We crossed the Apc^{min} mouse strain, a common intestinal tumour model, with Bmal1 mutant mice, which lack a functioning circadian clock. After creating an isogenic strain, we examined the number of tumours in control (*Bmal1*$^{+/+}$) and clock dead (*Bmal1*$^{-/-}$) animals. We derived organoids, a 3D cell culture method, from *Apc*$^{+/+}$*; Bmal1*$^{+/+}$ (healthy, clock-live), *Apc*$^{+/+}$, *Bmal1*$^{-/-}$(healthy, clock-dead), *Apc*^{min}*; Bmal1*$^{+/+}$(adenoma, clock-live), *Apc*^{min}*; Bmal1*$^{-/-}$ (adenoma, clock-live) mouse ileum and collected every 2h from 24-48h after synchronizing their circadian clock. Collected samples were sent for RNA sequencing and assessed for circadian regulated transcripts. This experiment was followed up by *in vitro* organoid assays.
Results: The circadian clock controls 41 genes in the intestinal epithelium, including genes like Tead4 which are known to be important in intestinal biology. There are twofold more tumours in *Bmal1*$^{-/-}$ mice than their *Bmal1*$^{+/+}$ littermates, and *Bmal1*$^{-/-}$ tumours upregulate Tead4 and Hippo pathway targets and downregulate Wnt pathway targets. *Bmal1*$^{-/-}$ adenoma organoids show increased self-renewal when compared to *Bmal1*$^{+/+}$ adenoma organoids. However, this increase in self-renewal is lost when organoids are treated with inhibitors of the hippo pathway.
Conclusions: The circadian clock is important in maintaining the health of an

organism, and disruption of the clock can lead to many health consequences including cancer. We show for the first time that the circadian clock controls the hippo signaling mediator *Tead4.* Additionally, we show that the loss of the clock leads to an increase in the number of tumours present in the epithelium which are characterized by an increase in hippo signaling. This research shows the important of considering time of day when studying stem cells during homeostasis and in cancer.

Funding Agencies: CIHRNSERC