The circadian clock in B16 melanoma cells controls their proliferation.

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Circadian disruption is associated with cancer. Cancer cells exhibit uncontrolled fast cell division. Since tumor suppressor and key cell cycle genes are regulated by circadian clocks, circadian dysfunction might be responsible for increased tumor proliferation. Here we addressed the possibility that improving circadian rhythms in tumor cells would control their cell cycle and thereby reduce proliferation.

First we characterized the circadian clock in B16 mouse melanoma cells. We were able to induce rhythmic gene expression with serum, forskolin and DEX in cultured Bmal1-luc or Per2-luc transfected B16 cells and repeated DEX treatment in vivo. Without continuous treatment rhythms quickly dampened and desynchronized in single cells, demonstrating a functional but unstable clock. Moreover, DEX treatment induced rhythmic expression of cell cycle genes and induced rhythmic entry into G0/1 and G2/M-phase. Interestingly, overall less DEX-treated cells entered the S-phase, indicating less DNA replication and proliferation.

To test whether melanoma cell growth is regulated by an intrinsic circadian clock, we measured the proliferation rate and apoptosis of cultured B16 melanoma cells after induction of circadian rhythms. With B16 cells in culture, two days after DEX treatment we counted about 50% fewer cells than without treatment and no change in apoptosis.

When B16 cells were injected into mice to generate subcutaneous tumors, clock gene expression in the tumors was low and arrhythmic, but repeated DEX treatment (every other day) induced circadian rhythms. Moreover, the DEX-treated tumors showed strongly reduced tumor growth in vivo.

Knockdown of Bmal1 in B16 cells using shRNA inhibited the effects of DEX treatment. Cell proliferation was undistinguishable from control shRNA treated cells demonstrating that the DEX effects rely on the induction of the circadian clock rather being a clock-independent action of DEX.

Together, these results indicate that the tumor clock can be manipulated to control cell cycle and tumor growth. This strategy might become a new, innovative way to slow down cancer progression and thereby improve the outcome of established anti-cancer therapies.