Studies on the modulation of cellular circadian clock by hormonal cycles, nutrition, and cellular crosstalk

楊, 丹

Name　：DAN YANG

Title　：Studies on the modulation of cellular circadian clock by hormonal cycles, nutrition, and cellular crosstalk
（細胞モデルを用いたホルモンのサイクル刺激、栄養、および細胞間クロストークによる概日時計の調節に関する研究）

Category：Kou

Thesis Summary

Animal studies have shown that irregular light-dark cycles or feeding-fasting cycles cause circadian desynchronization, and several nutrients have been identified as modulatory factors of the circadian clock. However, few studies have addressed the interaction effects of regular/irregular stimulation cycles of circadian signaling hormones or combination effects of nutrients with signaling hormones on the cellular clock in vitro. In addition, combined monitoring system of crosstalk between central and peripheral clock cells is lacking. This thesis addressed these points by using NIH3T3 cells and SCN2.2 cells transfected with Bmal1 promotor-driven Luciferase (Bmal1-Luc) reporter gene as peripheral and central clock models, respectively. Chapter 2 revealed that the Bmal1-Luc bioluminescence rhythms in NIH3T3 cells can be entrained to 22 and 24 h cycles during the cyclic dexamethasone stimulation period. Irregular dexamethasone treatment (16, 24, 16 h, sequentially; short-term jet lag protocol) resulted in an overall upregulation and phase shifts of temporal expression of several clock genes and cell cycle genes, including c-Myc and p53. Chapter 3 demonstrated that regular dexamethasone and insulin treatment (24 h cycles, 3 times) in NIH3T3 cells enhanced the cellular circadian rhythm without increases in c-Myc expression. Regular insulin treatment partially recovered the effect of irregular dexamethasone treatment on clock genes expression. Irregular insulin treatment alone had minimum effects on cellular clock, while it modified the effect of regular dexamethasone treatment. Chapter 4 demonstrated that a single pulse (15 min) of spermidine, a polyamine, strongly reset circadian phase advances in NIH3T3 cells in the presence or absence of dexamethasone. The effect was not blocked by RU486, an antagonist of glucocorticoid receptors, suggesting that spermidine acts through glucocorticoid receptor-independent pathways. Chapter 5 established a dual-color luciferase assay system of co-cultured SCN2.2 and NIH3T3 cells, which were transfected with Bmal1 promoter-driven green and red luciferases, respectively. In conclusion, the present thesis established a basis for studying the modulation of the cellular circadian clock by circadian hormonal/nutritional signals and crosstalk between central and peripheral clocks, and succeeded to identify spermidine as a potent modulator of the cellular clock..