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主　論　文　の　要　旨

論文題目　Study on biological clocks that underly various diseases
(様々な病気をもたらす生物時計に関する研究)

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論　文　内　容　の　要　旨

The circadian rhythm is an endogenous rhythm with a period of approximately 24 hours observed in almost all living things. The circadian clock regulates numerous physiological and behavioral processes including metabolism, hormone secretion, and sleep-wake cycles. The suprachiasmatic nucleus (SCN) is the central pacemaker of the circadian system, a bilateral structure located in the anterior part of the mammalian hypothalamus, that regulates peripheral circadian rhythms in the body. Importantly, most peripheral tissues and cells also contain self-sustained circadian clocks. The mammalian circadian clock involves a transcription-translation feedback loop.　Circadian locomotor output cycles kaput (CLOCK) and brain and muscle Arnt-like protein-1 (BMAL1) activate *Period* (*Per*) and *Cryptochrome* (*Cry*) genes, which then feedback and repress their transcription. There is increasing recognition of an essential link between circadian rhythm disorders and various diseases. Chronic disruption of the circadian clock is associated with many diseases, such as cardiovascular diseases, cancer, and immune and metabolic disorders. Therefore, it is crucial to develop circadian clock modulators to cure or prevent various diseases.

A drug repositioning approach is an effective approach for developing new therapeutic targets for existing drugs. In general, the development of new drugs, from drug discovery to market approval, is costly and time-consuming. In contrast, the drug repositioning approach enables shorter drug development cycles, lower cost, higher efficiency, and minimal risk of failure. Kampo medicine originated from Chinese medicine and gradually matured into a medical system unique to Japan. Kampo medicine has long been used to treat various diseases and is fully

integrated into the modern medical system. Crude drugs used in traditional Japanese Kampo medicine are an excellent source of new chemical entities for drug discovery. To identify new circadian clock modulators, I focused on Japanese Kampo medicine.

In the present study, I screened 137 crude drug extracts to act as circadian clock modulators in human U2OS cells stably expressing the clock reporter *Bmal1-dLuc*. I initially examined the effect of crude drugs on the circadian clock by measuring fluorescence oscillations of U2OS cells. This analysis identified 17 drugs that affect biological clock oscillations, mainly in terms of period and phase, suggesting that approximately 12 % of Kampo medicine modulates the circadian rhythm. The effects of hit candidates were validated by examining the dose-dependent effects on the circadian clock. To further investigate whether the effects of hit crude drugs were specific to reporter genes (*Bmal1-dLuc*) or cell types (U2OS cells), three representative hit crude drugs (Artemisiae Capillaris Flos, Perillae Herba, and Allii Chinense Bulbus) were examined in Rat-1 fibroblasts (*Per2-dluc*).

It is well known that all Kampo medicines are mixtures of multiple active ingredients. Therefore, active ingredients of Kampo medicines were examined in U2OS (*Bmal1-dLuc*). Interestingly, the most common targets for the hit active ingredients were AKT and its related pathways. Akt-related diseases mainly include cancer, neurological diseases, diabetes, cardiovascular diseases, inflammation, and autoimmune diseases. Although the involvement of Akt in the circadian clock has been reported in *Drosophila*, its involvement in the mammalian circadian system remained unclear. Therefore, I examined the effects of AKT activator SC79 and inhibitor A-443654 in U2OS cells. The AKT activator SC79 shortened the circadian period and advanced the phase at the highest dose. In contrast, the AKT inhibitor A-443654 shortened the circadian period at lower doses and lengthened the period at the highest dose. Three different AKT isoforms exist in mammals, namely AKT1, AKT2, and AKT3. To further explore the role of AKT in the circadian clock, siRNAs targeting *AKT1*, *AKT2*, and *AKT3* sequences were obtained. When I examined the effects of siRNA, triple knockdown of *AKT1*, *AKT2*, and *AKT3* shortened the circadian period.

Chronic disruption of the circadian clock due to shift work or travel across time zones has long-term consequences on human health. Due to the high cost and time-consuming nature of developing new drugs, drug repositioning approaches have become a popular and powerful approach. In the present study, I have identified circadian clock modulators from Japanese Kampo medicine. In addition, we have uncovered the involvement of the AKT signaling pathway in the regulation of the mammalian circadian system. Therefore, a drug repositioning approach using

Kampo medicines is a useful approach for identifying potential therapeutic treatments for chronic circadian disruption.