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Thesis/dissertation Title

Effect of cellular senescence on circadian clock properties in primary cultured human lung fibroblasts

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A circadian rhythm is any endogenous biological process that repeats itself every almost 24 hrs. These rhythms are generated by a system of clocks, called the circadian clocks, that are present in almost all cells of the body. To maintain order in the functioning of the system, a hierarchy exists in the clocks of this system. A master clock resides in the hypothalamic region of the brain, the suprachiasmatic nucleus (SCN), and this master clock regulates the function of all the other clocks in the body, which are termed as the peripheral clocks. This coordinated functioning between the master and peripheral clocks contributes to the optimum timing of all the cellular functions that ultimately gives rise to the rhythms observed at the cellular level, tissue level and finally at organismal level, elicited as the sleep-wake cycles, hormonal secretions, temperature rhythms etc. Hence, proper functioning of the circadian system is needed to maintain optimum health, as disruptions of circadian clocks lead to many pathological conditions.

Just like any other system, the circadian system has also been found to be affected by aging. The master clock SCN demonstrated prolonged period and delayed phases of the circadian gene expression patterns, both at the tissue level and at the cellular level. For the peripheral clocks, however, the results have been equivocal, with some tissues showing changes in the clock properties while some others do not. In addition, in all the studies, the peripheral tissues were collected from the intact animals and their circadian gene expression monitoring started immediately. As the peripheral clocks are under the regulation of the SCN in the intact animal, a major cause of the peripheral clock alterations were considered to be due to the aged SCN. Hence, whether the peripheral clocks also undergo intrinsic changes with aging is still unclear.

In my project, I sought to check whether the peripheral clocks also undergo intrinsic changes with aging, without the interference of SCN signals or any systemic cues that might obscure the results. To serve this purpose, I utilized the senescent human primary cultured cells as a cellular model of aging which conveniently avoided the effect of SCN signals or any other systemic cues. In recent years, senescent cells have gained widespread recognition as one of the basic drivers of the aging process. These cells are the permanently proliferation arrested

cells and they have been found to accumulate in the body with aging. Moreover, the senescent have also been found at the sites of many age-related pathologies and removal of these cells improved healthspan of the animals. Hence the senescent cells qualified as the *in vitro* model of aging. I serially passaged the proliferative cells until they reached replicative senescence and then compared the clock properties between the senescent and proliferative cells. I found that the replicative senescent cells possess the longer period and delayed phases, i.e. the altered circadian clock properties in comparison to their proliferative counterparts. To further validate the results, I used different resetting stimuli, namely dexamethasone and forskolin, to check whether the same pattern of results is obtained, and interestingly enough, the senescent cells invariably demonstrated the longer period and delayed phases, irrespective of the type of resetting stimulus used. This suggests that the alterations of the circadian clock are a general feature of the senescent cells.

Finally, to ensure that senescence is the underlying cause of these clock alterations, I used another approach to induce senescence. Instead of serial passaging to obtain the replicative senescent cells, I utilized hydrogen peroxide to induce premature senescence in the proliferative cells. The proliferative cells thus reached senescence in a significantly shorter span of time. These premature senescent cells also exhibited the longer period and delayed phases in comparison to the control proliferative counterparts. Thus, these results indicate that cellular senescence contributes to intrinsic circadian clock alterations. 3

My main objective was to check whether the peripheral clocks undergo intrinsic changes with aging at the cellular level. Using this *in vitro* model of aging, i.e. the senescent primary human cells, I found the peripheral clocks indeed undergo circadian clock alterations without the influence of the master clock SCN signals or any systemic cues. Hence my study at the cellular level builds upon previous studies, that in addition to the deficits of the aged master clock, alterations of the peripheral clocks, likely via senescence, also contributes to the age-related alterations of the circadian clock system. Thus investigation at the cellular level is a powerful and useful approach to dissect the molecular mechanisms of how aging alters the circadian clock. Further investigations using primary cells could shed light on the molecular mechanisms of aging in circadian clock, and perhaps help to promote healthy aging and longevity in the elderly.