Expression and Circadian Alteration of Clock Genes by Serum and Melatonin in breast epithelial and breast cancer cells

Shulin Xiang, Jun Dai*, Lulu Mao, Lin Yuan and Steven M. Hill, Department of Structural and Cellular Biology, Tulane Cancer Center Tulane University Health Sciences Center, New Orleans, LA 70112 *Cutaneous Biology Research Center, Massachusetts General Hospital, Charlestown, MA 02129

A growing body of knowledge is revealing the critical role of circadian physiology in the development of cancer. We have reported previously that human breast cancer cells have quit expressing the circadian clock gene, Per2. Re-expression of per2 can inhibit the growth and induce the apoptosis of MCF-7 breast cancer cells. Our aim in this study was to investigate the expression and circadian rhythm difference in clock gene between human mammary epithelial cell line MCF-10A and breast cancer cell MCF-7, and how the pineal hormone, melatonin, and the retinoid-related orphan receptor alpha-one (RORα1) influences 'clock' gene expression in both normal and malignant breast epithelial cells. For that purpose, we measured expression and circadian (rhythmic) changes in clock genes in MCF-10A breast epithelial and MCF-7 breast cancer cells in response to serum-shock. We demonstrate here that the clock genes Clock, Bmal1, Cry1, Cry2 are expressed in both MCF-10A and MCF-7 cells, whereas Per1 and Per2 genes are expressed in MCF-10A normal breast epithelial cells, but not in MCF-7 breast cancer cells. In response to serum shock the clock genes (Bmal1, Clock, Per1, Per2, Cry1, Cry2, Rev-Erb), and the clock associated genes (Sirt1 and MT1) show an oscillating expression pattern in MCF-10A cells, but no oscillation (very low expression levels) or a disrupted oscillation pattern with low amplitude in MCF-7 cells. However, the oscillatory pattern of ROR 1 is similar in both cell lines. We have found that ROR 1significantly enhances the transcription of Bmal1 in both MCF-10A and MCF-7 cells, that administration of melatonin suppresses the stimulatory effect of RORα1 on Bmal1 expression, and that luzindole, an MT1/MT2 melatonin-receptor antagonist, can reverse this effect. Knock-down of Per2 in MCF-10A cells, using an siRNA approach, results in a significant increase in the expression of Bmal1 and Clock genes and the oncogenic genes c-myc and Sirt1, while decreasing Per1, MT1, suggesting that the loss of Per2 in cells may promote the oncogenic process in breast epithelial cells. Ongoing studies seek to define the specific downstream signaling mechanism(s) triggered by melatonin that regulate Bmal1 expression and how this may affect other clock genes including Per1 and Per2 in human breast epithelial cells.

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