CLOCK GENES IN THE RAT MAMMARY GLAND

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The development and differentiation of the mammary gland, which plays a central role in its susceptibility to develop cancer, are regulated by pituitary and ovarian hormones that are, in turn, under the control of the hypothalamic suprachiasmatic nucleus (SCN). In addition, functional peripheral clock genes could affect mammary gland development via light-induced circadian disruption, and may increase its potential to develop malignancies. To investigate whether aging of the rats influences the mammary gland's gene expression in response to light stimuli, we designed this study for characterizing the pattern of expression of nine circadian rhythm-related genes in the mammary gland of virgin Sprague-Dawley rats at post-puberty and late adulthood.

For these experiments, one group of 50 and another of 100 day-old rats were exposed to standard 12 light:12 hr darkness photoperiod for two weeks. Then, four to five animals per group were euthanized every four hours at each one of the following circadian or Zeitgeber (ZT) times: ZT0 (lights on at 5:00 hr), ZT4, ZT8, ZT12 (lights off at 17:00 hr), ZT16, and ZT20. Mammary glands were dissected for RNA extraction and Real Time RT-PCR analysis of the following genes: *Period* (*Per*) *1*, *2*, and *3*, *Cryptochrome* (*Cry*) *1* and *2, basic helix-loop (bhlhb) 1 and 2,* brain and muscle ARNT-like protein (*Bmal1*) and *Clock.* Gene expression was expressed as the mean ± SD of four samples using β actin as an endogenous control.

The expression of *Bmal1*, B*hlhb2*, *Cry 2*, *Per1*, *Per 2*, *Per 3* and *Clock* genes was rhythmic during a 24 hour period in both age groups., The gene expression of *Cry 2* had a similar rhythmical fluctuation in both age groups, although in 50 day-old animals it showed a significantly higher expression at ZT3, ZT11 and ZT19 than the 100 day-old group. B*hlhb3* and *Cry1* gene expression did not show any relevant differences between the two age groups. Of great interest was our finding that the expression of the clock genes Bhlhb2 and Bmal genes exhibited overexpression at ZT23 (4am) in the 50 day-old group, whereas these genes had a very low expression level in the 100 day-old animals. These data suggest that in the rat mammary gland some of the circadian clock genes studied are affected by aging, and this in turn may influence the susceptibility to the rat mammary gland to carcinogenesis. (Study supported by NIEH grant UO1 ES012771 and grant supplement #16570)