Neoplasia. 2007 October; 9(10): 797–800. PMCID: PMC2040206

Expression of the Circadian Clock Genes *Per1* and *Per2* in Sporadic and Familial Breast Tumors[1]

Sherry L Winter,[*†] Lucine Bosnoyan-Collins,[*] Dushanthi Pinnaduwage,[‡] and Irene L Andrulis[*†§¶]
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Abstract

There is a growing body of evidence implicating aberrant circadian clock expression in the development of cancer. Based on our initial experiments identifying a putative interaction between *BRCA1* and the clock proteins Per1 and Per2, as well as the reported involvement of the circadian clock in the development of cancer, we have performed an expression analysis of the circadian clock genes *Per1* and *Per2* in both sporadic and familial primary breast tumors and normal breast tissues using real-time polymerase chain reaction. Significantly decreased levels of *Per1* were observed between sporadic tumors and normal samples ($P < .00001$), as well as a further significant decrease between familial and sporadic breast tumors for both *Per1* ($P < .00001$) and *Per2* ($P < .00001$). Decreased *Per1* was also associated with estrogen receptor negativity (53% *vs* 15%, $P = .04$). These results suggest a role for both Per1 and Per2 in normal breast function and show for the first time that deregulation of the circadian clock may be an important factor in the development of familial breast cancer. Aberrant expression of circadian clock genes could have important consequences on the transactivation of downstream targets that control the cell cycle and on the ability of cells to undergo apoptosis, potentially promoting carcinogenesis.

Keywords: Circadian clock, gene expression, Per1, Per2, *BRCA1*

Introduction

Deregulation of the circadian clock has been implicated in many types of cancers, and large studies indicate that increased breast cancer risk is correlated with increased years of night shifts [1–3]. The circadian clock proteins Per1 and Per2 function in a series of positive and negative feedback loops that coordinate the circadian clock in both the brain and peripheral tissues [4]. Molecularly, several links between the circadian clock and DNA damage response have been drawn. Mice that are deficient in the *mPer2* gene are not only deficient in their circadian clock rhythm but also cancer-prone and sensitive to γ-irradiation [5]. Additionally, thymocytes from *mPer2* mutant mice either are less sensitive or have a slower apoptotic response to γ-irradiation [6]. Conversely, overexpression of *mPer2* induces apoptosis and alters the expression levels of apoptosis-related genes in mouse tumor cells [7].

Moreover, overexpression of *Per1* sensitizes HCT116 colon cancer cells to infrared-induced DNA apoptosis, and suppression of *Per1* blunts apoptotic response [8]. Per1 physically interacts with ATM and Chk2, which are known to function in a DNA damage response complex along with other components such as *BRCA1*. Furthermore, *Per1* expression is decreased in small lung cell carcinoma tumors, as well as in a small cohort of breast tumors [8].

Based on the reported involvement of the circadian clock in the development of several cancers, including breast cancer, as well as on experiments that have identified a putative interaction between BRCA1 and Per1 and Per2 in a yeast two-hybrid system, we used real-time polymerase chain reaction (PCR) to examine *Per1* and *Per2* in both familial and sporadic breast tumors and normal breast tissues to identify any changes in gene expression. We observed significant alterations in *Per1* and *Per2* expression suggesting that their deregulation may contribute to the development of sporadic or familial breast cancer, or both. Additionally, we analyzed the associations of *Per1* or *Per2* with breast tumor characteristics and found a significant association of *Per1* expression with estrogen receptor (ER) status. Per1 and Per2 influence the transcription of genes and induce apoptosis; therefore, lack or misregulation of their expression could contribute to an improper apoptotic response and the development of breast cancer.

Materials and Methods

Tumor Samples

mRNA from 34 sporadic breast tumors were obtained as part of a prospective study of molecular alterations in auxiliary node-negative disease [9]. Eleven tumors were from familial breast cancer cases from the Ontario site of the Breast Cancer Family Registry [10].

DNA and RNA have previously been extracted by conventional techniques [11]. mRNA from normal breast tissue samples were obtained from 13 additional subjects with “sporadic” breast cancer. The normal breast tissue samples were selected by a pathologist from regions adjacent to tumors. All relevant institutional review boards approved the study protocol, and written informed consent was obtained from all participants.

Real-Time PCR Reactions

cDNA was reverse-transcribed, and real-time PCR was carried out as described [12]. *HPRT-1*, which has little variation in expression between tumors, was chosen for normalization. Primer/probe pairs used for the experiments were purchased from ABI (Foster City, CA) [*BRCA1*, cat no. HS 00173233-m1; *Per1*, cat no. HS 00242988-m1; *Per2*, cat no. HS 002561440m1; *HPRT-1*, cat no. 4326321E (endogenous control)].

Statistical Analysis

A descriptive analysis comparing the frequency distributions of tumor characteristics between the *Per1* and *Per2* groups (high *versus* low) was performed by a pathologist using contingency tables. The association of each characteristic with *Per1* or *Per2* expression was investigated by

Fisher's exact test [13]. The high-*versus*-low levels of *Per1* or *Per2* expression were established by dividing the tumor sets around the mean level of expression of each gene.

All statistical analyses were performed using SAS Statistical Software, version 8.2 (SAS, Inc., Cary, NC). R statistical software, version 1.9.1, was used to generate box plots. $P < .05$ was considered to be statistically significant for all analyses.

Results

Expression of *Per1* and *Per2* in Breast Tumors

Our preliminary results identifying the interaction of BRCA1 with Per1 and Per2 in the yeast two-hybrid system (data not shown) and the increasing amount of evidence that circadian clock perturbations may result in breast cancer led us to investigate the expression levels of *BRCA1*, as well as *Per1* and *Per2*, in both sporadic and familial primary human breast tumors and normal breast tissues. Not surprisingly, low levels of *BRCA1* mRNA were identified in sporadic tumors compared to both normal breast tissues and familial cancers, consistent with decreased expression, rather than mutation, being the means for *BRCA1* inactivation in these tumors (Table 1) [14].

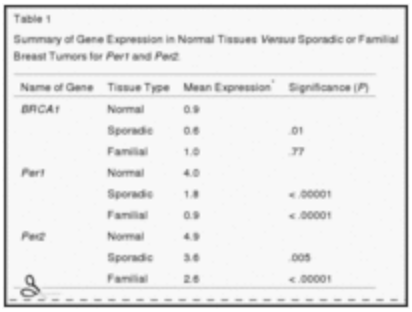

Table 1

Summary of Gene Expression in Normal Tissues *Versus* Sporadic or Familial Breast Tumors for *Per1* and *Per2*

Name of Gene	Tissue Type	Mean Expression*	Significance (*P*)
BRCA1	Normal	0.9	
	Sporadic	0.6	.01
	Familial	1.0	.77
Per1	Normal	4.0	
	Sporadic	1.8	< .00001
	Familial	0.9	< .00001
Per2	Normal	4.9	
	Sporadic	3.6	.005
	Familial	2.6	< .00001

Table 1
Summary of Gene Expression in Normal Tissues *Versus* Sporadic or Familial Breast Tumors for *Per1* and *Per2*.

Importantly, both *Per1* and *Per2* exhibit significantly decreased levels of expression in sporadic and familial tumors compared to normal breast tissues (Table 1). Furthermore, *Per1* expression is also significantly decreased in familial tumors compared to sporadic tumors (Table 1), suggesting that deregulation of the circadian clock may contribute to the familial aspect of these tumors.

Association of *Per1* and *Per2* with Sporadic Tumor Characteristics

The use of primary human breast tumors allowed us to examine correlations between tumor or disease characteristics with *Per1* and *Per2* expression levels, and the results for *Per1* are summarized in Table 2. The group of breast tumors with a "low" level of *Per1* or *Per2* mRNA was compared to those with "high" *Per1* or *Per2* expression levels, and we detected a weak association between low *Per1* expression and ER status ($P = .04$), which needs to be investigated

further in a larger sample set. Tumors with low *Per1* levels were more likely to be negative for ER receptor than those with high *Per1* levels (53% *vs* 15%). This association was not observed for *Per2* ($P = .30$), and no other significant associations were identified for *Per2* (not shown).

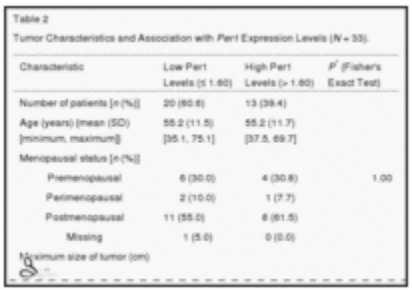

Table 2

Tumor Characteristics and Association with *Per1* Expression Levels ($N = 33$).

Discussion

Deregulation of the circadian clock may lead to the development of several types of cancer, including breast cancer. Consequently, we performed a genetic analysis of *BRCA1* and *Per1* and *Per2* in both sporadic and familial primary breast tumors and normal breast tissues to identify aberrations in their expression levels and to analyze whether changes in *Per1* and *Per2* expression are associated with breast tumor characteristics. Interestingly, both *Per1* and *Per2* exhibit significantly decreased levels of expression in both sporadic and familial tumors compared to normal breast tissues. Additionally, the expression of *Per1* was significantly decreased in familial breast tumors compared to its expression in sporadic specimens, suggesting that a possible deregulation of the circadian clock could contribute to the familial aspect of these tumors. Our results identifying decreased *Per1* and *Per2* expression in tumors is supported by previous work showing that *Per* gene deregulation is caused by methylation of the *Per1* or *Per2* promoter in approximately 50% of analyzed breasts cancers in Taiwanese women [15]. Per1 and Per2 regulate transcription and promote apoptosis; therefore, decreased levels of *Per1* and *Per2* in breast tumors could have important ramifications on the expression of both circadian and other downstream genes and could potentially limit the apoptotic response of the cell.

The use of primary human breast tumors allowed us to examine correlations between tumor or disease characteristics and *Per1* and *Per2* expression levels. Interestingly, we observed a statistically significant association between low levels of *Per1* expression and negative ER status, and a weak association between low levels of *Per1* expression and tumor size using our small sample, which need to be investigated further in a larger sample set. ER levels are maintained by a number of mechanisms, including activation or repression of the ER promoter at the transcriptional level. Interestingly, tumors with *BRCA1* mutations also tend to be negative for ER expression [16,17], and it is possible that Per1 acts with proteins such as *BRCA1* to regulate ER transcription.

We have analyzed the expression levels of *Per1* and *Per2* in both sporadic and familial breast tumors, as well as in normal breast tissues, and have determined that there are significant decreases in *Per1* and *Per2* expression levels in these tumors compared to normal breast tissues.

Additionally, we identified a further decrease in *Per1* expression when comparing tumors from women with familial breast cancer to tumors from women with sporadic breast cancer, suggesting that circadian clock disruption may contribute to the inherited form of the disease. Furthermore, we have detected a significant association of decreased *Per1* levels with ER-negative breast tumors. These results suggest a role for both Per1 and Per2 in normal breast function and the potential deregulation of *Per1* and *Per2* expression in breast cancer development.

Abbreviations

Per
: period

ER
: estrogen receptor

BRCA1
: breast cancer susceptibility gene

Footnotes

[1]We gratefully acknowledge the support of the National Cancer Institute of Canada with funds from the Terry Fox Run (I.L.A.) and a predoctoral fellowship from the United States Army Medical Research and Materiel Command (DAMD017-02-1-0496; to S.L.W.).

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