L. Zheng¹, Y.J. Seon¹, J. McHugh²,
S. Papagerakis^{3*}, and
P. Papagerakis^{1*}

¹Department of Orthodontics and Pediatric Dentistry, School of Dentistry, University of Michigan, Ann Arbor, MI, USA; ²Department of Pathology, Medical School, University of Michigan, Ann Arbor, MI, USA; and ³Department of Otorhinolaryngology, Medical School, University of Michigan, Ann Arbor, MI, USA; *corresponding authors, petrosp@umich.edu and silvanap@umich.edu

J Dent Res 91(8):783-788, 2012

ABSTRACT

Circadian rhythms are endogenous self-sustained oscillations with 24-hour periods that regulate diverse physiological and metabolic processes through complex gene regulation by “clock” transcription factors. The oral cavity is bathed by saliva, and its amount and content are modified within regular daily intervals. The clock mechanisms that control salivary production remain unclear. Our objective was to evaluate the expression and periodicity of clock genes in salivary glands. Real-time quantitative RT-PCR, *in situ* hybridization, and immunohistochemistry were performed to show circadian mRNA and protein expression and localization of key clock genes (*Bmal1*, *Clock*, *Per1*, and *Per2*), ion and aqua channel genes (*Ae2a*, *Car2*, and *Aqp5*), and salivary gland markers. Clock gene mRNAs and clock proteins were found differentially expressed in the serous acini and duct cells of all major salivary glands. The expression levels of clock genes and *Aqp5* showed regular oscillatory patterns under both light/dark and complete-dark conditions. *Bmal1* overexpression resulted in increased *Aqp5* expression levels. Analysis of our data suggests that salivary glands have a peripheral clock mechanism that functions both in normal light/dark conditions and in the absence of light. This finding may increase our understanding of the control mechanisms of salivary content and flow.

KEY WORDS: circadian clock, saliva, transcriptional regulation, circadian rhythms, *Aqp5*, expression pattern.

DOI: 10.1177/0022034512451450

A supplemental appendix to this article is published electronically only at <http://jdr.sagepub.com/supplemental>.

Received February 21, 2012; Last revision May 16, 2012; Accepted May 20, 2012

© International & American Associations for Dental Research

Clock Genes Show Circadian Rhythms in Salivary Glands

INTRODUCTION

The oral cavity has a moist environment due to saliva, which is secreted from numerous major and minor glands in the oral cavity (Tucker and Miletich, 2010). The major, parotid, sublingual, and submandibular salivary glands (SGs) normally contribute over 90% of the total volume of unstimulated saliva (Edgar, 1990). SGs contain several cell types, including acinar cells, which are responsible for water and protein secretion, myoepithelial cells surrounding the acini and ducts, and ductal cells, which modulate the composition of the saliva (Gresik, 1994; Denny *et al.*, 1997).

Saliva contains numerous enzymes, hormones, growth factors, immunoglobulins, and many other active molecules (Dawes, 1969; Levine, 1993). It has been shown that salivary flow rate follows circadian rhythms (Dawes, 1972). The secretion levels of salivary substances also follow circadian rhythms, which seem to be important for effective nutrition and self-defense (Dawes, 1974). These studies suggest that the SGs may contain a circadian clock to regulate the type, amount, and content of saliva. Indeed, the existence of such a clock mechanism has been demonstrated for other vital organs such as the kidney (Stow and Gumz, 2011). SGs and kidneys share physiological mechanisms involving a multitude of ion and water channels. Several genes that regulate fluid movement are highly expressed in both kidneys and SGs (Takata *et al.*, 2004; Maeda *et al.*, 2008). Little is known about the molecular mechanisms that control circadian saliva production and secretion. This study was undertaken to fill this important scientific gap.

A circadian clock is regulated by differential daily expression of 20 transcription factors called clock genes. There is a central clock in the brain and several peripheral clocks that can also act independently of the central clock. The central clock is composed of about 20,000 neurons, all of which express clock genes that oscillate in synchrony (Welsh *et al.*, 1995; Ikeda, 2004). Clock genes are defined by a set of criteria that include rhythm in activity or amount as well as molecular evidence of a feedback mechanism (Williams and Sehgal, 2001; Myers *et al.*, 2003). Clock genes transmit output signals that drive rhythms of gene expression in central and peripheral tissues. The most direct mechanism by which clock genes drive circadian gene expression is through regulation of promoter activity of clock-controlled genes. Clock genes can also indirectly drive circadian gene expression through regulation of promoter activity of clock-controlled genes, which in turn regulate the transcription of downstream genes at specific daily times (Kondratov *et al.*, 2006).

Very recently, clock genes have begun to be discovered as key regulators of several diverse diseases, including cancer and diabetes (Krugluger *et al.*, 2007; Mostafaie *et al.*, 2009; Marcheva *et al.*, 2010; Xia *et al.*, 2010; Shimba

Figure 1. Clock gene RNAs (*Per1*, *Per2*, *Bmal1*, and *Clock*) were detected in both mouse SGs and the kidney. The SG marker (*Smgc*) was expressed only in SGs. SGs, salivary glands; kid, kidney; NC, negative control.

et al., 2011). Saliva plays numerous protective roles for oral tissues maintenance (Mandel, 1989; Dowd, 1999). Adequate salivary flow and saliva content are directly related to health status. In contrast, reduced salivary flow rate is an indicator of xerostomia, which may be caused by SG disorders, such as Sjögren's syndrome, and chronic sialoadenitis (Daniels and Fox, 1992; Mese and Matsuo, 2007). Reduced salivary flow is also found in cases of sialolithiasis, due to blockage of the ducts, and in post-radiation head and neck cancer patients (Rice, 1984; Mese and Matsuo, 2007). Many aspects of the biological mechanisms that result in xerostomia remain unclear. Regardless of the cause, patients with hyposalivation suffer from difficulties with swallowing and food mastication, dental caries, hampered speech, impaired taste, and nocturnal oral discomfort (Vissink *et al.*, 2003a,b). Our study aims to increase understanding on how clock genes affect salivary flow rates and content. This knowledge may be applied in the future toward a better understanding of salivary gland physiology.

MATERIALS & METHODS

Animals and Circadian Experiments

Male C57BL/6J mice (from 8 to 12 wks of age) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Only male mice were used, to minimize potential sex-related differences in hormonal differences over circadian rhythms. The animal use was approved by the Animal Welfare Committee of the University of Michigan. For circadian experiments, all time interval calculations were based on the indicated zeitgeber (ZT, an event that provides the settings for a biological clock), and 6:00 a.m. was considered ZT 0 (detailed in the Appendix).

Human Tissues

Samples of human salivary glands were obtained from patients of the University of Michigan Hospital (Ann Arbor, USA) with

prior signed informed consent form. All the material used for this study was collected from biopsies necessary for the diagnosis of salivary gland pathologies. The study was conducted in accordance with IRB regulations.

RNA Isolation and RT-PCR

Total RNA was isolated from freshly excised submandibular SGs and kidney tissues at the same time-point, with the use of TRIzol (Invitrogen, Carlsbad, CA, USA), and a 2- μ g quantity of RNA was reverse-transcribed with TaqMan reverse transcription reagents (Applied Biosystems, Branchbury, NJ, USA). The submandibular gland is a mixed gland that secretes the highest amount of unstimulated saliva and contains both serous and mucous cells, making the submandibular gland a good model for evaluating the differential expression of clock RNAs and proteins in salivary glands. The resulting cDNA was then amplified by RT-PCR or real-time RT-PCR (detailed in the Appendix) with specific primers (Appendix Table 2).

In situ Hybridization, Immunohistochemistry, and Imaging

In situ hybridization (ISH) and immunohistochemistry (IHC) of SG sections were performed (described in the Appendix). Briefly, paraffin mouse submandibular, parotid, and sublingual salivary glands were used. At the end, sections were photographed on an Olympus microscope. Sense probes of mouse *Bmal1* and *Per2* were used as negative controls of ISH. As IHC negative controls, both omission of primary antibodies and omission of secondary antibodies were used.

Cell Culture and Transfection Studies

HEK293 (human embryonic kidney 293) cells were plated in 6-well culture plates at 70% confluence and transfected with 2 μ g of *Bmal1*-pCMV (kindly provided by Dr. Lei Yin, Department of Molecular and Integrative Physiology, University of Michigan) or empty pCMV plasmid with Lipofectamine 2000 (Invitrogen; detailed in the Appendix). After transfection, RNA was reverse-transcribed into cDNA and used for quantitative RT-PCR.

Statistical Analysis

Statistical analyses were performed with Student's unpaired *t* test. Each experiment was performed at least twice, and the representative data are presented as means $\pm$ SD of at least 3 independent replicates.

RESULTS

Detection Clock RNAs by RT-PCR

Aryl hydrocarbon receptor nuclear translocator-like (*Arntl* or *Bmal1*), clock homolog (mouse) (*Clock*), period homolog 1 (*Drosophila*) (*Per1*), and period homolog 2 (*Drosophila*) (*Per2*)

mRNAs were detected in mouse submandibular SG extracts by conventional RT-PCR (Fig. 1), as well as in the kidney, which is a tissue known to be regulated by clock genes (positive control). However, submandibular gland protein C (*Smgc*), a specific marker of SGs, can be detected only in SGs but not in the kidney. All the PCR products were sequenced to confirm clock RNA expression.

Localization of Clock Gene RNAs and Proteins in SGs

In situ hybridization results showed that *Bmal1* and *Per2* RNAs were detected strongly in the nucleus and cytoplasm of striated ducts and mucous acini, but weakly in serous acini cells (Figs. 2M, 2N). No positive staining was identified with a sense probe by *in situ* hybridization (data not shown). A similar expression pattern was found for clock proteins with IHC staining of mouse parotid (Figs. 2A, 2D, 2G, 2J), sublingual (Figs. 2B, 2E, 2H, 2K), and submandibular (Figs. 2C, 2F, 2I, 2L) SG sections. Four key clock proteins, *BMAL1*, *CLOCK*, *PER1*, and *PER2*, were found to be rarely expressed in serous acini, but their expression in mucous acini and striated ducts was very strong in submandibular SGs. In contrast, all 4 clock proteins were very strongly expressed in serous acini and mucous acini, as well as in striated ducts. We also tested human submandibular SG sections for *PER1* expression. As in the mouse sections, strong expression was found in human mucous acini and striated ducts (Fig. 2O). Among the 4 clock proteins studied here, *CLOCK* and *PER2* showed stronger expression levels than *BMAL1* and *PER1*. Negative control sections (omission of the first antibody) showed absence of staining (data not shown).

Clock Genes Are Expressed in a Circadian Manner in Mouse SGs

We used real-time PCR to assess whether intestinal clock genes are expressed in a rhythmic circadian manner in mouse SGs under regular light/dark cycles. Rhythmic expression patterns were observed for two consecutive days for all clock RNAs studied in the SGs (Figs. 3A, 3C, 3E, 3G). *Bmal1* and *Clock*

Figure 2. Clock gene products localization in SGs. Immunohistochemistry results showed that *BMAL1* (A-C), *CLOCK* (D-F), *PER1* (G-I), and *PER2* (J-L) proteins were expressed in the nucleus of both acinar cells and in the epithelial cells of the ducts in mouse SGs. Expression was much stronger in duct cells. The same expression pattern was detected in human SGs (O). *Bmal1* and *Per1* RNAs were detected in the nuclear of serous acini and duct cells of mouse SGs by *in situ* hybridization (M, N). MA, mucous acini; SA, serous acini; SD, striated ducts; PSG, parotid salivary gland; SLSG, sublingual salivary gland; SMSG, submandibular salivary gland. Bars = 50 μ m in A, B, D, E, G, H, J, K; = 20 μ m in C, F, I, L, M-O.

showed highest expression levels at ZT 0 and lowest at ZT 12. In contrast, *Per1* and *Per2* RNAs showed strong expression levels at ZT 12 and low expression at ZT 0. The same expression pattern of individual clock gene RNAs was apparent on day 2 as on day 1, confirming the presence of a 24-hour cycle.

To determine whether rhythmically expressed clock genes in the SGs were driven by the light-dark cycle, we kept the mice in constant darkness to eliminate the effect of light, with *ad libitum* access to food. Under dark/dark conditions, although clock gene RNAs showed reversed circadian expression, they

Figure 3. Analysis of real-time PCR data showed that *Clock*, *Bmal1*, *Per1*, and *Per2* RNAs are expressed in a rhythmic circadian manner in SGs under light/dark and dark/dark conditions (A-H). The mRNA levels are expressed as means of SE ($n = 3$ mice per time-point). All time interval calculations are based at the indicated zeitgeber (ZT, an event that provides the settings for a biological clock), and 6:00 a.m. was considered ZT 0.

still maintained rhythmic expression patterns in the SGs (Figs. 3B, 3D, 3F, 3H). Under dark/dark conditions, the circadian amplitude was decreased for *Per1* and *Per2* but not for *Bmal1* and *Clock*.

Downstream Targets of Clock Genes

We then investigated whether clock genes regulate aquaporin 5 (*Aqp5*), solute carrier family 4 (anion exchanger), member 2 (*Slc4a2* or *Ae2a*), and carbonic anhydrase 2 (*Car2*) expression. By promoter sequence analysis, we found an E-box putative binding site on the promoter of *Aqp5* and a RRE-box putative binding site on the *Car2* promoter (Appendix Table 1). Preferential binding of clock transcription factors has been shown on both E-box and RRE-box promoter sequences. In contrast, we did not find a putative clock-binding site on the *Ae2a* gene promoter. We then investigated whether clock genes regulated *Aqp5*, *Ae2a*, and *Car2* expression in SGs *in vitro*.

We then tested if the expression of key functional genes, *i.e.*, *Aqp5*, *Car2*, and *Ae2a*, follows a circadian pattern in SGs.

Under light/dark conditions, *Aqp5* and *Ae2a* showed the same rhythmic expression patterns as clock genes (Figs. 4A, 4C). In contrast, *Car2* expression did not follow a circadian pattern (data not shown). Of interest, under dark/dark conditions, only *Aqp5*, but not *Ae2a*, showed a reproducible circadian pattern (Figs. 4B, 4D, respectively). We focused our subsequent studies on *Aqp5*.

HEK293, which is a Human Embryonic Kidney cell line, was used for the transfection experiments. We performed real-time quantitative RT-PCR to evaluate whether clock genes and *AQP5* are expressed in HEK293 cells. Our results showed that, as in SGs, HEK293 cells also express *BMAL1*, *CLOCK*, *PER1*, *PER2*, and *AQP5* (data not shown). After *Bmal1* overexpression by transfection (Fig. 4E) and cell-cycle synchronization, up-regulated *AQP5* expression levels were found in HEK293 cells (Fig. 4F). Of interest, *AQP5* up-regulation also followed a circadian pattern (Fig. 4F).

DISCUSSION

Clock genes, which generate circadian rhythms, are expressed in a circadian manner in the suprachiasmatic nucleus of the brain and in various peripheral tissues. Here, we showed that clock gene RNAs and clock proteins were differentially detected in SG cells. Especially strong expression was detected in serous

acinar and duct cells, which indicates that clock genes may play important roles in salivary flow and salivary electrolyte flux regulation. This is the first detailed characterization of clock genes and their products, by *in situ* hybridization and immunohistochemistry, in all 3 major SGs.

To confirm the existence of circadian regulation in SGs, we then evaluated the oscillatory profiles of key clock gene RNAs, including *Per1*, *Per2*, *Clock*, and *Bmal1*, in submandibular glands. All clock genes studied here showed robust circadian expression rhythms in SGs when mice were housed under 12:12-hour light-dark (L/D) cycle conditions. Our data are consistent with those from a previous study which reported the expression of clock genes in submandibular glands (Furukawa *et al.*, 2005). In addition to that study, our study also characterized, for the first time, the cellular localization of clock proteins in all 3 major SGs. More importantly, our study demonstrated that clock gene oscillation persists even in dark-dark conditions (D/D), suggesting that the circadian clock mechanism of SGs may also function independently of the central clock located in the brain. These findings provide a major advancement in our knowledge of SG physiology.

Previous studies showed that clock genes are the key regulators of circadian gene expression and daily physiological functions in many organs (Huang *et al.*, 2011; Lefta *et al.*, 2011). However, the molecular mechanisms that control circadian salivary secretion are unclear. We hypothesized that the SGs contain a peripheral clock that regulates their functions through differential expression of clock genes, the genes responsible for maintaining circadian rhythms. This hypothesis is in accordance with our data showing that clock genes continue to be expressed at regular daily intervals, even under D/D conditions. We also postulated that clock genes may regulate the expression of key SG genes such as *Aqp5*, *Ae2a*, and *Car2*. Our promoter analysis revealed that 2 out of 3 key SG genes (*i.e.*, *Aqp5* and *Car2*) contain putative promoter binding sites for clock protein binding, supporting a regulatory role for clock genes in SGs. Consistently, *Aqp5* expression showed a clear circadian pattern under both normal light/dark conditions and under complete-dark conditions. Furthermore, *Bmal1* overexpression resulted in *AQP5* RNA up-regulation, suggesting that *Aqp5* is a key target of clock genes in SGs. In contrast, *Ae2a* and *Car2* did not show a definitive circadian pattern of expression, suggesting that these 2 genes may not be direct targets of clock genes in SGs.

The saliva secreted by the SGs fulfills many functions to maintain the normal homeostasis of the oral cavity (Dawes, 1969). The major constituent of saliva is water. The SGs also secrete ions, mainly K(+) and HCO₃(-), and re-absorb Na(+) and Cl(-). The volume and the composition of the oral fluid can vary during the day within regular time intervals and within every individual. Salivation can be either stimulated or reduced by several factors and diseases. Hyposalivation, for example, is directly related to high caries, xerostomia, and pain, and much research is focused on treating salivary abnormalities. However, the direct links between causes and effects in different salivary pathologies are unclear. Recently, abnormal expression of clock genes has been found in patients with reduced salivary flow (our own unpublished observations). We therefore postulate that a clock mechanism that implies direct or indirect regulation of key genes important for SG physiology may be altered in diseases with abnormal salivary flow. We are currently evaluating the role of clock genes in salivary gland pathologies.

In conclusion, oscillations of clock genes were first detected in the SGs under light/dark but also under complete-dark conditions. Analysis of our data supports the idea that the SGs contain a circadian clock mechanism. Understanding the role of clock genes in controlling salivary flow and electrolyte content

Figure 4. Expression of potential downstream targets of clock genes. Analysis of real-time PCR data showed that *Aqp5* RNA is expressed in a circadian manner in SGs under light/dark and dark/dark conditions (A, B). In contrast, *Ae2a* showed a circadian pattern only in light/dark conditions (C) but not in complete-dark conditions (D). The *AQP5* mRNA level in HEK293 was up-regulated after *Bmal1* overexpression. All time interval calculations were based at the indicated zeitgeber (ZT 0 was considered to be 2 hrs after cell-cycle synchronization). The experiments were repeated 3 times, and 1 representative experiment is presented (**p* < 0.05; compared with 0 hr).

will enhance our understanding of the regulation of salivary functions and will provide a foundation for subsequent studies that may elucidate the potential links between clock genes and salivary diseases.

ACKNOWLEDGMENTS

This research was supported by funds provided by the Department of Orthodontics and Pediatric Dentistry, School of Dentistry, University of Michigan; NIH/NCI cancer center core grant P30 CA46592 (start up funds to SP); and NIH/NIDCR grant DE 018878 (PP). The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

- Daniels TE, Fox PC (1992). Salivary and oral components of Sjögren's syndrome. *Rheum Dis Clin North Am* 18:571-589.
- Dawes C (1969). The effects of flow rate and duration of stimulation on the concentrations of protein and the main electrolytes in human parotid saliva. *Arch Oral Biol* 14:277-294.
- Dawes C (1972). Circadian rhythms in human salivary flow rate and composition. *J Physiol* 220:529-545.

- Dawes C (1974). Rhythms in salivary flow rate and composition. *Int J Chronobiol* 2:253-279.
- Denny PC, Ball WD, Redman RS (1997). Salivary glands: a paradigm for diversity of gland development. *Crit Rev Oral Biol Med* 8:51-75.
- Dowd FJ (1999). Saliva and dental caries. *Dent Clin North Am* 43: 579-597.
- Edgar WM (1990). Saliva and dental health. Clinical implications of saliva: report of a consensus meeting. *Br Dent J* 169:96-98.
- Furukawa M, Kawamoto T, Noshiro M, Honda KK, Sakai M, Fujimoto K, *et al.* (2005). Clock gene expression in the submandibular glands. *J Dent Res* 84:1193-1197.
- Gresik EW (1994). The granular convoluted tubule (GCT) cell of rodent submandibular glands. *Microsc Res Tech* 27:1-24.
- Huang W, Ramsey KM, Marcheva B, Bass J (2011). Circadian rhythms, sleep, and metabolism. *J Clin Invest* 121:2133-2141.
- Ikeda M (2004). Calcium dynamics and circadian rhythms in suprachiasmatic nucleus neurons. *Neuroscientist* 10:315-324.
- Kondratov RV, Shamanna RK, Kondratova AA, Gorbacheva VY, Antoch MP (2006). Dual role of the CLOCK/BMAL1 circadian complex in transcriptional regulation? *Faseb J* 20:530-532.
- Krugluger W, Brandstaetter A, Kallay E, Schueller J, Krexner E, Kriwanek S, *et al.* (2007). Regulation of genes of the circadian clock in human colon cancer: reduced period-1 and dihydropyrimidine dehydrogenase transcription correlates in high-grade tumors. *Cancer Res* 67:7917-7922.
- Lefta M, Wolff G, Esser KA (2011). Circadian rhythms, the molecular clock, and skeletal muscle. *Curr Top Dev Biol* 96:231-271.
- Levine MJ (1993). Development of artificial salivas. *Crit Rev Oral Biol Med* 4:279-286.
- Maeda S, Kuwahara S, Ito H, Tanaka K, Hayakawa T, Seki M (2008). Expression and localization of aquaporins in the kidney of the musk shrew (*Suncus murinus*). *J Histochem Cytochem* 56:67-75.
- Mandel ID (1989). The role of saliva in maintaining oral homeostasis. *J Am Dent Assoc* 119:298-304.
- Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, *et al.* (2010). Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* 466:627-631.
- Mese H, Matsuo R (2007). Salivary secretion, taste and hyposalivation. *J Oral Rehabil* 34:711-723.
- Mostafaie N, Kallay E, Sauerzapf E, Bonner E, Kriwanek S, Cross HS, *et al.* (2009). Correlated downregulation of estrogen receptor beta and the circadian clock gene Per1 in human colorectal cancer. *Mol Carcinog* 48:642-647.
- Myers EM, Yu J, Sehgal A (2003). Circadian control of eclosion: interaction between a central and peripheral clock in *Drosophila melanogaster*. *Curr Biol* 13:526-533.
- Rice DH (1984). Advances in diagnosis and management of salivary gland diseases. *West J Med* 140:238-249.
- Shimba S, Ogawa T, Hitosugi S, Ichihashi Y, Nakadaira Y, Kobayashi M, *et al.* (2011). Deficient [sic] of a clock gene, brain and muscle Arnt-like protein-1 (BMAL1), induces dyslipidemia and ectopic fat formation. *PLoS One* 6:e25231.
- Stow LR, Gumz ML (2011). The circadian clock in the kidney. *J Am Soc Nephrol* 22:598-604.
- Takata K, Matsuzaki T, Tajika Y (2004). Aquaporins: water channel proteins of the cell membrane. *Prog Histochem Cytochem* 39:1-83.
- Tucker AS, Miletich I (2010). Salivary glands: development, adaptations and disease. 1st ed. Basel: S. Karger Publishers.
- Vissink A, Jansma J, Spijkervet FK, Burlage FR, Coppes RP (2003a). Oral sequelae of head and neck radiotherapy. *Crit Rev Oral Biol Med* 14:199-212.
- Vissink A, Burlage FR, Spijkervet FK, Jansma J, Coppes RP (2003b). Prevention and treatment of the consequences of head and neck radiotherapy. *Crit Rev Oral Biol Med* 14:213-225.
- Welsh DK, Logothetis DE, Meister M, Reppert SM (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* 14:697-706.
- Williams JA, Sehgal A (2001). Molecular components of the circadian system in *Drosophila*. *Annu Rev Physiol* 63:729-755.
- Xia HC, Niu ZF, Ma H, Cao SZ, Hao SC, Liu ZT, *et al.* (2010). Deregulated expression of the Per1 and Per2 in human gliomas. *Can J Neurol Sci* 37:365-370.