Normalized coefficient of variation (nCV): a method to evaluate circadian clock robustness in population scale data

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Abstract

Summary: Robust oscillation of clock genes is a core feature of the circadian system. Relative amplitude (rAMP) measures the robustness of clock gene oscillations but only works for longitudinal samples. We lack a method for estimating robust oscillations from human samples without labeled time. We show that the normalized coefficient of variation (nCV) of 10 clock genes is linearly correlated with their normalized rAMP, independent of time labels. We found that the mean nCV of clock genes are consistently decreased in tumors compared to nontumors, suggesting a new therapeutic target in cancer treatment by enhancing clock robustness. nCV can provide a simple measure of the clock robustness in population-level datasets.

Availability and implementation: The nCV package (https://github.com/gangwug/nCV) and web application (https://github.com/gangwug/nCVapp) are available on the GitHub repository.

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Supplementary information: Supplementary data are available at Bioinformatics online.
epithelial cells (MCF10A; rAMP = 0.085) compared to breast cancer cells (MCF7; rAMP = 0.003) (Fig. 1A, left panel). The rAMP calculation requires the sample collection time (Fig. 1A, right panel). However, the vast majority of patient datasets available do not have recorded collection times. Other metrics, such as the clock correlation distance (Shilts et al., 2018), can indicate clock function in the absence of collection time, but cannot measure clock gene robustness (Supplementary Fig. S1). Therefore, we need a way to estimate clock gene robustness in population data where sampling time is not recorded.

To address this, we selected a group of 10 clock genes that: (i) are important in circadian regulation, (ii) cycle in multiple tissues, (iii) are phased across the full circadian cycle and (iv) represent a range of rAMP values (Supplementary Fig. S2). Then, we searched for a time-independent measure of variance that is linearly correlated with rAMP. Median absolute deviation (MAD), standard deviation (SD) and the CV are common measures of transcript level correlation distance (Shilts et al., 2018) to nrAMPs in 12 mouse tissues (Fig. 1C; r = 0.96 and P = 2.4e−13) (Chen et al., 2016) and human skin samples ordered by CYCLOPS (Fig. 1D; r = 0.96 and P = 2.4e−13) (Wu et al., 2020a). In sum, nCV is a strong surrogate measure for nrAMP even when sample collection time is unknown.

The vast majority of human datasets do not have time of day information available. This includes most cancer datasets. Thus, we applied nCV to study clock gene robustness in tumor versus adjacent nontumor samples without labeled time. The majority of clock genes showed reduced nCV in tumor samples from patients with hepatocellular carcinoma, lung adenocarcinoma, clear cell renal cell carcinoma, breast invasive carcinoma and thyroid carcinoma (Supplementary Fig. S6). The nCV of ARNTL and PER2 is consistently decreased in tumor samples from all eight tested datasets. In sum, clock robustness is reduced in tumors compared to nontumors in all eight datasets (Wilcoxon test, P = 0.007; Fig. 1E).

Studies report inconsistent changes in clock gene expression between different human cancer types (Savvidis and Koutsilieris, 2012; Ye et al., 2018). However, we show that clock robustness using nCV is consistently reduced across human cancers. Clock robustness may in fact be an important therapeutic target in cancer. For example, enhancing the circadian clock function in cancer cells inhibits tumor growth (Kiessling et al., 2017). Beyond cancer, nCV can be used to estimate clock robustness in any context where population scale data are available. In sum, nCV provides a straightforward measure of circadian clock function in the absence of labeled time, the vast majority of all human datasets.
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Data availability

The datasets were derived from sources in the public domain: [GEO, https://www.ncbi.nlm.nih.gov/geo/] and [FireBrowse, http://firebrowse.org/].

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