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Network Analysis of Effect of Light-Dark Time Ratio on the Gene Expression Profile of Mouse Skin

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Abstract

Introduction: Circadian rhythms refer to daily cyclic events such as activity and rest in biology. A protein-based core related to the mechanism of circadian is identified. In the present study, the gene expression profiles of mouse skin in different conditions of light-dark times were investigated via protein-protein interaction (PPI) analysis to explore the main affected genes.

Methods: GSE174155 was derived from Gene Expression Omnibus (GEO) and was analyzed via GEO2R to find the significant differentially expressed genes (DEGs). The gene expression profiles of Cry-null (genotype: cryptochrome-1(-/-): crytochrome-2 (-/-)) mouse skin versus the wild-type samples in the various circadian times (CTs) were assessed. The queried DEGs plus 50 first neighbors were included in a PPI network via the STRING database by Cytoscape software. The networks were analyzed and the central nodes were evaluated.

Results: Three groups of mice based on CTs were identified. 15, 15, and 14 central nodes were determined as central nodes for the analyze networks. There was not a common central node for the analyzed networks.

Conclusion: It was pointed out that the light/dark time ratio had a gross effect on the gene expression profile of the skin in the mice. Results imply more investigations to suggest a standard protocol related to CT, considering human lifestyle and exploring suitable protective methods for the jobs which are fixed in the abnormal CT sets.

Keywords: Light; Circadian; Gene; Network; Mice.

Introduction

Chronobiology is the field that includes basic mechanisms of the biological timekeeping systems (the high-frequency daily cycles, monthly or annual cycles) and the resulting potential penalties of their disappointment. Circadian rhythms are, the daily cycles and circadian is rooted from the Latin phrase meaning "about day". Rest and activity are well-known circadian rhythms. Investigations indicate that external time signals, such as the light-dark cycle, can harmonize or drag circadian rhythms.[1] It is pointed out that the circadian clock is changed to join in external environmental changes and internal physiology. Much research has revealed that the perturbation of circadian rhythms affects human health and arises the risk of several diseases including metabolic disorders, cardiovascular diseases, and cancer.[2]

It has been reported that the circadian clock synchronizes biology with the 24-hour light-dark of the earth. This timekeeping mechanism is based on largely conserved proteins.[3] Cryptochrome-1 (Cry-1) and cryptochrome-2 (Cry-2) are mammalian proteins that belong to the family of plant blue-light receptors (cryptochromes). Cry-1 and

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Cry-2 are known as core clock components.[4,5] Due to the importance of protein roles in the circadian mechanism, the proteomic evaluation of circadian events has attracted researchers' attention.[6] Like proteomics, gene expression investigation is applied to explore various aspects of the circadian clock mechanism.[7] Protein-protein interaction (PPI) network analysis as a valuable tool is used to analyze proteomic and genomic findings.[8] In a PPI network analysis, the genes or proteins connect to each other by the links to form a network. Each element of the PPI network can be characterized by its centrality properties such as degree, betweenness centrality, closeness centrality, and stress.[9,10] Gene expression changes depend on circadian activity and are investigated by researchers in a variety of samples from plants to animals.[11,12] In the present study, the gene expression profiles of mice under different conditions of light-dark times were investigated via PPI network analysis to find the critical dysregulated genes in response to the applied conditions. The correlation between the circadian time (CT) change and the gene expression alteration could be assessed.

Materials and Methods

As it is presented in the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE174155), GSE174155 contains 12 samples (GSM5288069-80) of mouse skin epidermis from foot pads. The gene expression profiles of 6 wild types and 6 Cry-null mice at six CTs (CT0, CT4, CT8, CT12, CT16, and CT20) are presented in GSE174155. Since CT is an important variable,[13] we assessed a combination of gene expression profiles of wild-type skin and Cry-null samples at different CTs to explore the importance of CTs. An evaluation was done in 3 ranges of CTs including (CT0 and CT4), (CT8 and CT12), and (CT16 and CT20).

We identified the significant differentially expressed genes (DEGs) for each analysis considering $P<0.01$ and fold change > 2. The significant DEGs plus 50 added first neighbors were included in a network via the "protein query" of the STRING database by Cytoscape software. The first neighbor genes were added to maximize connections between the queried DEGs. The networks were analyzed by "Network Analyzer" to find the hub nodes. The Top 10% of queried DEGs based on the degree value were determined as hub nodes. The hub nodes of the 3 assessments were compared to explore relationships between the evaluated gene expression profiles. 10 top added first neighbors in each analysis were selected as first neighbor hub nodes. The common first neighbor hubs for the three analyses were identified.

Results

For the first assessment (comparison of gene expression profiles of CT0-CT4 of the Cry-null and wild types), among 183 significant DEGs, 147 individuals were recognized via the STRING database. To maximize the number of connections between the queried DEGs, we added the 50 first neighbors to the DEGs, and the network was constructed. The network including 147 queried DEGs and 50 added first neighbors was analyzed, and 10% of the top queried nodes were selected as hubs based on the degree value. The identified hub nodes and the related centrality parameters are shown in Table 1.

In a similar performance, 188 significant DEGs were determined to discriminate the CT8-CT12 Cry-null samples from wild-type individuals. 151 DEGs among the 188 significant DEGs were recognized by STRING database. The 151 recognized DEGs (by the STRING database) plus 50 added first neighbors formed a network. The related hubs of this network are presented in Table 2.

The third network was formed by 137 recognized DEGs (among 167 significant queried DEGs) for CT16-CT20 analysis. The central nodes of this investigation are presented in Table 3.

Since the first neighbor genes can be important players besides the queried DEGs, 10 first neighbor hub nodes were identified for each analysis. The common hubs for the three assessments were determined. The common hubs and the related ranks and degree values in the analyses are shown in Table 4.

Discussion

The application of PPI network analysis to explore the molecular mechanism of many biological events and diseases is an attractive activity and method in progress.[14] Singh et al published a document about using the PPI network to evaluate the circadian rhythm pathway in Camellia sinensis.[15] In the present study, the gene expression profiles of two Cry-null mouse skin samples

Table 1. Hub Nodes of CT0-CT4 Analysis

Gene	Degree	Betweenness Centrality	Closeness Centrality	Stress
Flt3	34	0.037	0.478	8172
Il10	34	0.002	0.487	1114
Pik3cd	34	0.005	0.479	2306
Cd28	32	0.002	0.470	778
Rock1	30	0.036	0.470	8444
Tfrc	28	0.022	0.466	4498
Cdk14	24	0.003	0.463	984
Mycn	22	0.006	0.445	1846
Bub1b	20	0.001	0.456	362
Ccl3	20	0.008	0.427	2224
Camk2a	19	0.019	0.430	3990
Rnasel	18	0.000	0.420	18
Cxcl9	17	0.017	0.421	3546
Kalrn	17	0.002	0.424	560
Nedd4l	14	0.013	0.444	2088

Data are ranked based on degree values.

as a group with an adjacent range of CT were compared with the wild types. As it is shown in Tables 1-3, there are 15 or 14 central DEGs which discriminate the Cry-null mice from the reference wild-type samples. Surprisingly, the central genes for examination in the three ranges of CTs are different. It can be concluded that CT alteration induces a gross effect on the gene expression profiles of samples. The previous studies have discussed the importance of light time effect on the circadian rhythm of gene expression and chromatin accessibility.[16]

Table 2. Hub Nodes of CT8-CT12 Analysis

Gene	Degree	Betweenness Centrality	Closeness Centrality	Stress
Itgb1	56	0.055	0.571	8908
Pxn	48	0.008	0.550	2858
Pik3cg	46	0.007	0.559	3414
Fgf2	43	0.003	0.529	1216
Itgb2l	37	0.003	0.472	1208
Ntrk3	35	0.004	0.482	1012
Gngt1	26	0.013	0.458	2164
Fgf18	21	0.000	0.439	50
Cdc25a	17	0.004	0.446	1896
Cdk14	17	0.005	0.442	2288
Rap2a	17	0.017	0.431	3450
Il6st	16	0.000	0.419	74
Cx3cl1	13	0.000	0.423	8
Nfatc2	13	0.000	0.423	56
Tnc	12	0.000	0.408	38

Data are ranked based on degree values.

Table 3. Hub nodes of CT16-CT20 analysis

Gene	Degree	Betweenness Centrality	Closeness Centrality	Stress
Itgav	38	5E-03	0.510	1712
Mapk8	32	1E-02	0.508	1840
Cd34	30	1E-03	0.489	462
Itga4	30	2E-03	0.469	542
Cd80	29	2E-02	0.474	2968
Cybb	29	4E-03	0.485	1238
Reln	25	9E-03	0.474	1534
Nox4	24	2E-03	0.471	764
Git2	19	5E-04	0.445	284
Adam10	18	1E-03	0.471	300
Icam2	16	7E-05	0.411	36
Synpo2l	16	3E-02	0.423	9888
Cdkn1c	14	9E-04	0.446	214
Spry2	13	2E-03	0.439	342

Data are ranked based on degree values.

As it is shown in Table 1, the 3 top central genes for the lowest CT range are FMS-like tyrosine kinase 3 (Flt3), interleukin 10 (Il10), and phosphatidylinositol 3-kinase catalytic delta polypeptide (Pik3cd) which are characterized by logFC -3.42, 3.47, and -4.99 respectively. Results of an investigation indicated that the inhibition of FLT3 whose level arose in acute lymphoblastic leukemia patients led to induced pronounced apoptotic responses in an antileukemic activity.[17] Il10 is known as an important immunoregulatory cytokine.[18] It is pointed out that Pik3cd is involved in cell growth and invasion of colorectal cancer.[19]

The middle CT range is characterized by the 3 top central DEGs of integrin beta 1 (fibronectin receptor beta) (Itgb1), paxillin (Pxn), and phosphoinositide-3-kinase, catalytic, gamma polypeptide (Pik3cg). Amounts of logFC for Itgb1, Pxn, and Pik3cg is recorded as 2.85, 2.44, and -3.58, respectively. The up-regulation of Itgb1 during the development and metastasis of renal cell carcinoma is reported by Erdem m et al.[20] Yuan et al published a document about the overexpression of Pxn and the promotion of hepatocellular carcinoma.[21] Pik3cg is another catalytic polypeptide of phosphoinositide 3-kinase. The delta catalytic polypeptide of this enzyme was discussed in the lowest CT range analysis.

Integrin alpha V (Itgav), mitogen-activated protein kinase 8 (Mapk8), CD34 antigen (Cd34), and integrin alpha 4 (Itga4) (it has the same degree value as Cd34) appear as central genes of the highest circadian range assessment (see Table 3). LogFC values for Itgav, Mapk8, Cd34, and Itga4 are reported as 3.33, 3.83, -3.63, and 2.71. The up-regulation of Itgav in nasopharyngeal carcinoma has been investigated by Huang et al.[22] Assessments showed that Mapk8 is involved in corneal pain syndrome in severe dry eye disease.[23] An evaluation revealed that FLT3 and mutations of nucleophosmin induce the down-regulation of CD34 in blasts of patients with acute myelogenous leukaemia.[24]

The overall conclusion consists of the complex role of CT in the regulation of gene expression of the treated samples. Flt3 as the potent central DEGs in the lowest CT range assessment is not included among the significant DEGs of the other two analyses. Integrin sub-

Table 4. The Common Hub Nodes Among the Added First Neighbor Genes for the Three Analyses

Gene	$Rank_{CT0-CT4}$	$Degree_{CT0-CT4}$	$Rank_{CT8-CT12}$	$Degree_{CT8-CT12}$	$Rank_{CT16-CT20}$	$Degree_{CT16-CT20}$
Ctnnb1	2	60	2	61	5	58
Akt1	4	56	9	56	1	66
Src	7	53	1	63	2	61
Egfr	9	52	4	58	6	56

family appears as key up-regulated DEGs in the middle and highest CT evaluations. However, Itga9 is down-regulated in the lowest CT analysis and is not pointed as a central gene. It seems each CT plays its role in a complex manner.

As it is shown in Table 4, there are 4 common first neighbor central genes among the ten hub nodes of each analysis. Catenin beta 1 (Ctnnb1) which is characterized by a degree value of about 60 for all assessments is involved in melanoma and early brain metastases.[25] The role of Ctnnb1 in cell development and differentiation is highlighted in the report by Ma et al.[26]

Conclusion

Our evaluation revealed that each CT or balance of light/dark time plays a unique role in the gene expression profile of the studied organism. The outcome of this analysis shows that crucial functions of an organism are affected by CT. Two suggestions can be concluded from the finding; the first suggestion is to find a standard protocol related to CT considering human lifestyle to protect human health, and the second one is to explore supplementary protective methods for the jobs which are fixed in the abnormal CT sets.

Conflict of Interests

The authors declare that they have no conflict of interest.

Ethical Considerations

This project was approved by Shahid Beheshti University of Medical Sciences (ethical code: IR.SBMU.RETECH.REC1401.484).

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