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研究課題名（和文）Modulation of circadian clock and its therapeutic implications in invasive breast carcinoma

研究課題名（英文）Modulation of circadian clock and its therapeutic implications in invasive breast carcinoma

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研究成果の概要（和文）：希少糖であるD-アロースを用いた薬理試験を行い、マウスおよびヒト乳がん細胞において、細胞増殖を抑制することを明らかにした。また、遺伝子発現データから、D-アロース処理後は、ビヒクルやD-グルコース処理に比べ、Per1、Per2、Cry2の発現が増加することがわかった。また、これまでのデータをもとに、概日リズムの遺伝子オントロジーに属する遺伝子とglut1との相関を解析した。興味深いことに、D-アロースを投与すると、HDAC1およびHDAC2遺伝子の発現が減少することが示された。したがって、これらのデータは、概日時計の調節が浸潤性乳癌の治療アプローチとなりうるという我々の仮説と一致する。

研究成果の学術的意義や社会的意義
Alteration of clock genes is highly associated with cancer cell proliferation and metastasis. Therefore, our findings indicated that modulating the circadian clock could be a remedial approach for attenuating metastasis as well as improved outcomes in patients with invasive breast carcinoma.

研究成果の概要（英文）：Our data indicated that Per1 and Per2 mRNA expression decreased in breast carcinoma cells compared to normal mammary tissue in mice. Since glucose metabolism has a significant impact on clock genes, we conducted pharmacological studies with rare sugar, D-allose. Cell proliferation was reduced after treatment with D-allose in mouse and human breast carcinoma cells. The gene expression data revealed that Per1, Per2 and Cry2 expression were increased after treatment with D-allose compared to the vehicle or D-glucose treatment. Based on our previous data, we analyzed the correlation of glut1 with those genes belong to the gene ontology of circadian rhythm. Interestingly, our data indicated that treatment with D-allose reduced the HDAC1 and HDAC2 gene expression compared to the control or equimolar D-glucose. Therefore, these data are in line with our hypothesis that modulation of circadian clock might be a potential therapeutic approach for invasive breast carcinoma.

研究分野：Cancer metabolism

キーワード：breast carcinoma　metastasis　circadian clock

１．研究開始当初の背景

Breast cancer is the second most common cancer and the fifth most common cause of cancer related deaths worldwide [Ferlay J et al. Int J Cancer. 2015, 136:E359–E386]. Regardless of a drastic improvement of diagnosis and therapeutic intervention, annual incidence of breast cancer has tripled over the past two decades in Asia. Indeed, the development of intrinsic chemoresistance is one of the most important hindrances underlying poor outcomes with the conventional therapeutic interventions in clinics [Abdullah LN et al. Clin Transl Med. 2013, 2:3]. Therefore, it is an urgent need to develop novel therapeutics by exploring new targets, which might treat breast cancer.

Intrinsic circadian clocks are driven by environmental time cues, which then translate into molecular oscillations within individual cells. These cell-autonomous molecular oscillators (core member of circadian clock) make up the body's internal timing system, and are involved in the synchronization of clock-controlled physiological processes by determining the expression levels of clock-controlled genes [Rahman A et al. Int J Mol Sci. 2018, 19(2), pii: E400]. At molecular level, circadian rhythms are generated by a system of interlocking autoregulatory transcriptional/translational feedback loop. However, alteration in molecular circadian rhythm is highly associated with the tumor progression in breast cancer [Cadenas C et al. Cell Cycle. 2014, 13:20, 3282-3291]. Previous reports demonstrated that over-expression of Per2 in colon cancer results in cell cycle arrest, growth inhibition and apoptosis, while Per2 mutants exhibit dysregulation of c-Myc, and its target cell-cycle regulators cyclin D1 and Gadd45 in cancer cells [Kelleher FC et al. Cancer Lett. 2014, 342:9-18]. Similarly, inhibition of Per1 alters the expression of key cell cycle regulators, while Per1 overexpression can reduce these in colon, lung and breast cancer cells [Gery S et al. Mol Cell. 2006; 22:375-82]. Moreover, silencing of Clock [Hoffman et al. Cancer Res. 2010, 70(4):1459-68] and overexpression of Bmal1 [Zeng ZL et al. J Biochem. 2010, 148(3):319-26] genes resulted in attenuation of the proliferation of breast cancer cells.

The above mentioned background information suggest that alteration of clock genes is highly associated with cancer cell proliferation, however, little information has been reported in connection with the invasive breast carcinoma. Therefore, based on our recent data and analysis of publicly available data, we hypothesized that modulating the circadian clock might be a remedial approach for attenuating metastasis as well as improved outcomes in patients with invasive breast carcinoma.

２．研究の目的

Since modulation of the clock genes has a great impact on cancer cell proliferation and progression, in this study, we would like to investigate the detailed molecular mechanisms underlying the breast cancer metastasis. Moreover, we would also like to explore the molecular targets, which might involve in the clock genes-mediated breast cancer metastasis, and subsequently would make efforts to identify specific ligands which may help to normalize the alteration of circadian clock in patients with invasive breast carcinoma for a better prognosis

３．研究の方法

a) Human metastatic breast cancer cell line (MDA-MB-231) [Rezwan A et al. NPJ Breast Cancer. 2015, 1:15017] as well as mouse metastatic breast cancer cell line (4T1) were used throughout the study.

b) Overexpression of PER1 and PER2 was conducted by pharmacological intervention with D-allose.

c) After gene manipulation, cell proliferation (WST-1) assay as well as mRNA expression of clock genes were measured by real time PCR analysis.

e) To identify the specific targets, gene analysis in the TCGA database was conducted.

f) Finally, identified gene expression was measured in D-allose-treated breast carcinoma cells.

４．研究成果

First, we measured the mRNA expression of core clock genes in mouse breast cancer cells (4T1) and mouse healthy mammary tissue. Quantitative real time PCR data revealed that the mRNA expression of Per1 and Per2 was significantly lower in breast carcinoma cells compared to the normal mammary tissue (Figure 1).

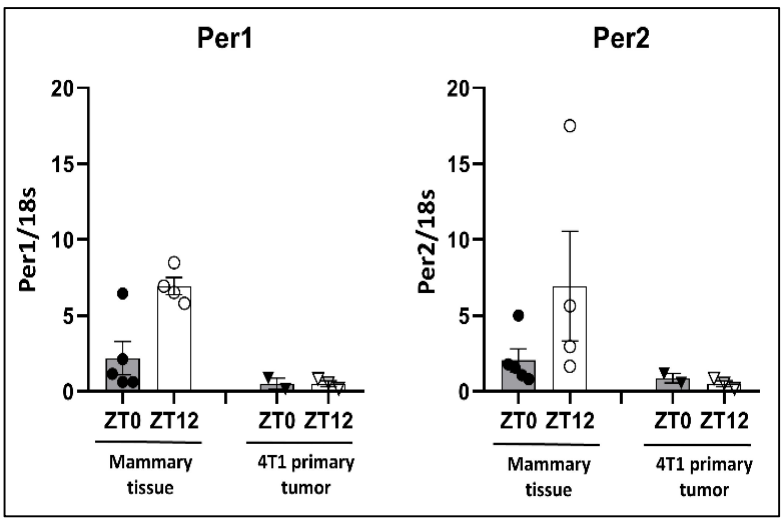

Figure 1. mRNA expression of Per1 and Per2 in mouse mammary tissues and breast carcinoma (4T1) cell xenograft tissues.

Since glucose metabolism has a significant impact on clock genes, before going to the knockdown or over-expression studies, we conducted pharmacological studies with rare sugar, D-allose.

Accumulating evidences have indicated that d-allose reduces glucose uptake through downregulation of glut-1. Therefore, we have checked the cell proliferation in mouse (4T1) and human (MDA-MB-231) breast carcinoma cells and found that d-allose reduced the cell proliferation (Figure 2A, B). Following intervention with d-allose (50 mM) for 48 hrs, quantitative real time PCR was performed to check the mRNA expression of core clock genes. The gene expression data revealed that Per1, Per2 and Cry2 expression were increased after treatment with D-allose compared to the vehicle or D-glucose treatment (Figure 2C-H), which might be associated with reduced cell proliferation in breast cancer cells. Therefore, these data are in line with our hypothesis that modulation of circadian clock might be a potential therapeutic approach for invasive breast carcinoma. Based on our previous data, we made efforts to analyze the correlation of glut1 with those genes belong to the gene ontology of circadian rhythm in human invasive breast carcinoma TCGA

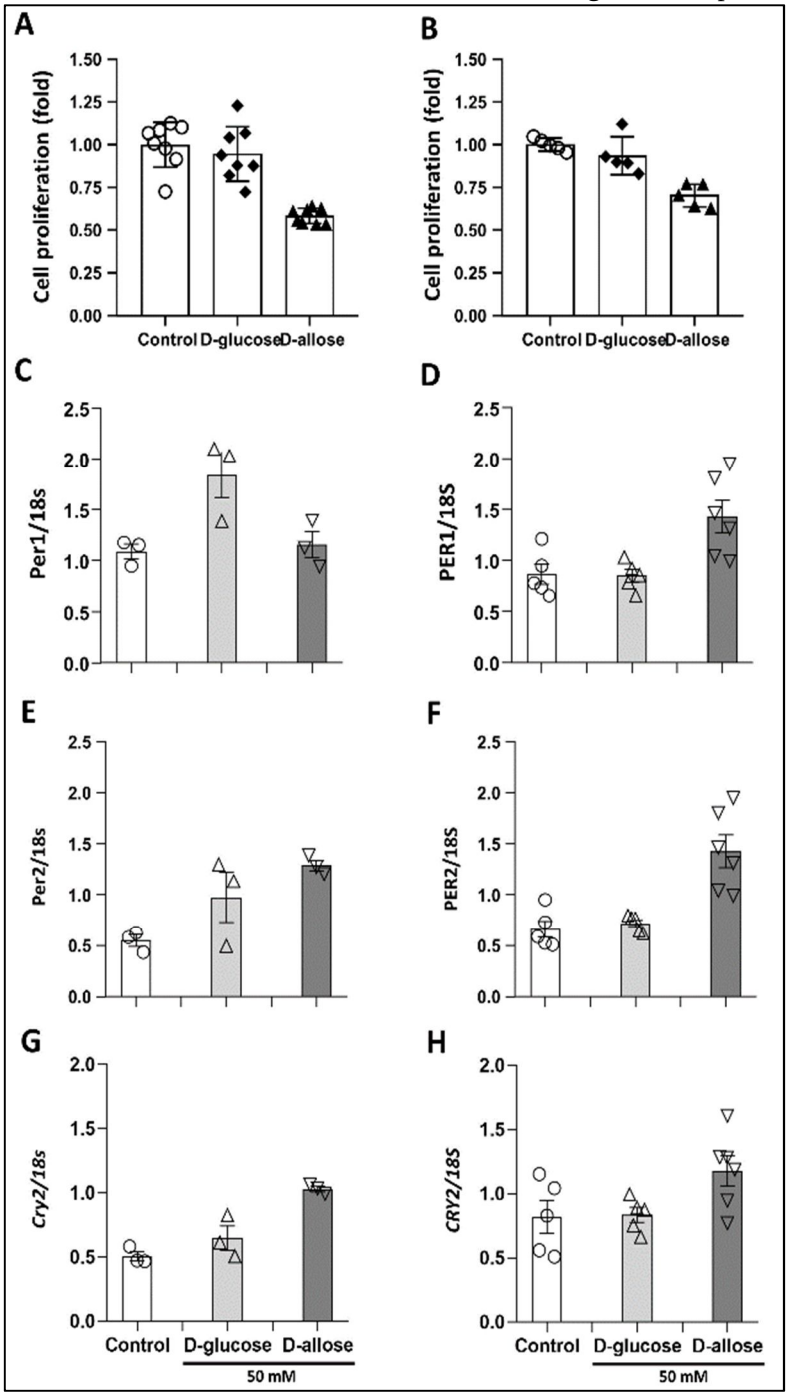

Figure 2. Cell proliferation following treatment with D-allose in mouse breast carcinoma, 4T1 cells (A) and human breast carcinoma cells, MDA-MB-231 (B). Circadian clock genes mRNA expression of Per1 and Per2 in mouse mammary tissues and breast carcinoma cells (4T1). Circadian clock genes namely Per1, per2 and cry2 mRNA expression in in 4T1 cells (C, E, G) and MDA-MB-231 cells (D, F, H).

database. Remarkably we found that the top five negatively correlated genes with glut1 (SLC2A1) were Cry2, RORC, Sirt1, OGT and Per2, where as top five positively correlated genes were HDAC2, HDAC1, PPP1CB, LGR4 and NAMPT (Figure 3).

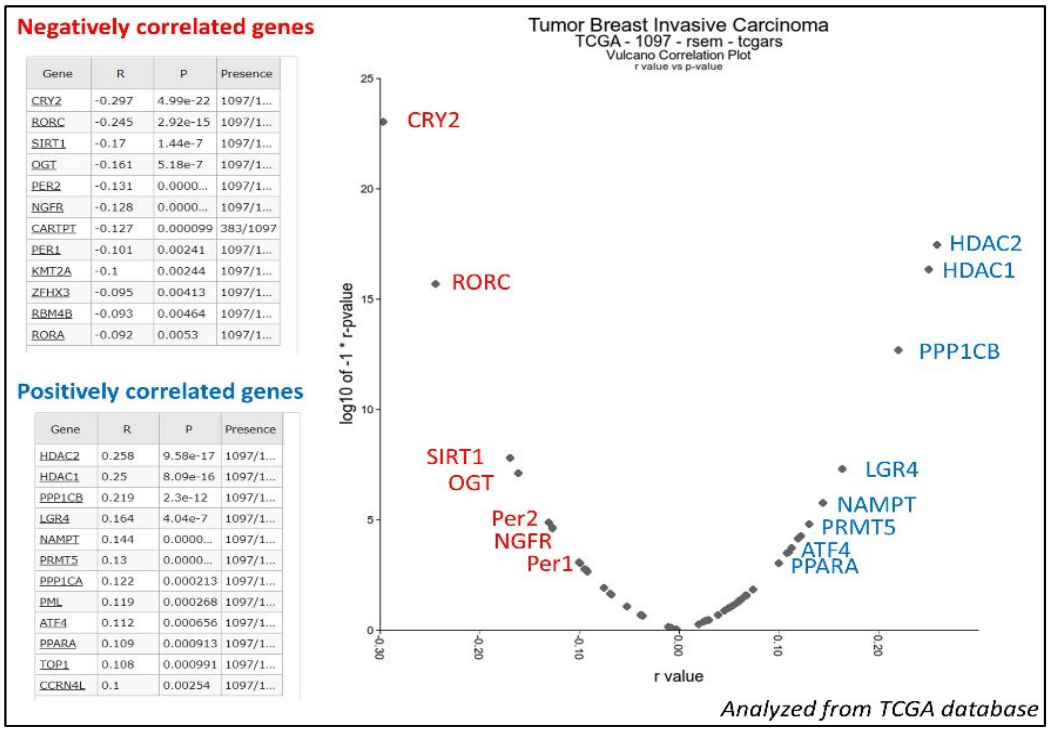

Figure 3. Analysis of the correlation of glut1 with those genes belong to the gene ontology of circadian rhythm in human invasive breast carcinoma TCGA database.

Therefore, based on the findings from TCGA analysis, we checked the alteration of genes associated with histone acetylation during the modulation of circadian clock in mouse breast carcinoma cells. Interestingly, our data indicated that treatment

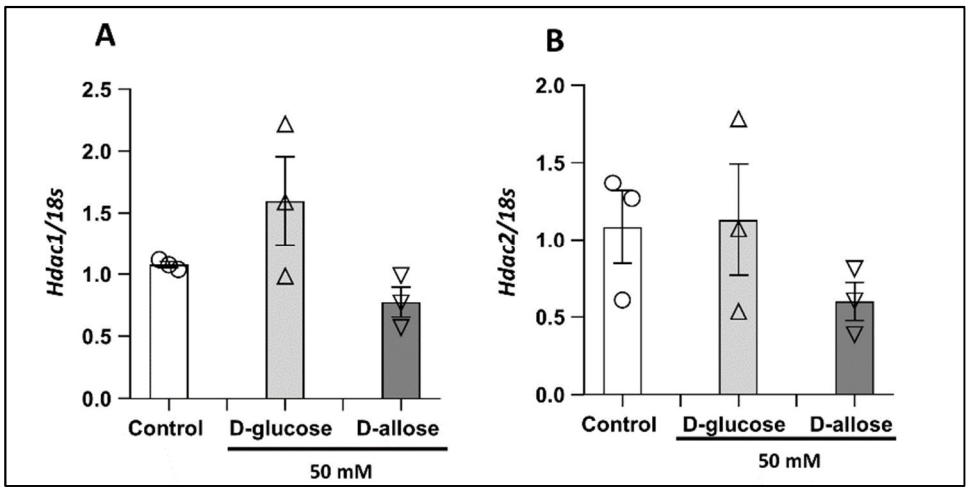

Figure 4. mRNA expression of Hdac1 (A) and Hdac2 (B) in mouse invasive breast carcinoma, 4T1 cells.

with D-allose reduced the HDAC1 and HDAC2 gene expression compared to the control or equimolar D-glucose (Figure 4). These findings suggest us, alteration in glucose uptake could be associated with the modulation of circadian clock genes in association with the modification in the histone acetylation.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Sumaya Akter, Akhi Moni, Golam Mahbub Faisal, Muhammad Ramiz Uddin, Nourin Jahan, Md Abdul Hannan, Asadur Rahman, Md Jamal Uddin	４．巻 19
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３．雑誌名 Int J Environ Res Public Health	６．最初と最後の頁 1864
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/ijerph19031864.	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関