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Data in Brief

Transcriptomic profiling of human embryonic stem cells upon cell cycle manipulation during pluripotent state dissolution

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ABSTRACT

While distinct cell cycle structures have been known to correlate with pluripotent or differentiated cell states [1], there is no evidence on how the cell cycle machinery directly contributes to human embryonic stem cell (hESC) pluripotency. We established a determinant role of cell cycle machineries on the pluripotent state by demonstrating that the specific perturbation of the S and G2 phases can prevent pluripotent state dissolution (PSD) [2]. Active mechanisms in these phases, such as the DNA damage checkpoint and Cyclin B1, promote the pluripotent state [2]. To understand the mechanisms behind the effect on PSD by these pathways in hESCs, we performed comprehensive gene expression analysis by time-course microarray experiments. From these datasets, we observed expression changes in genes involved in the TGFβ signaling pathway, which has a well-established role in hESC maintenance [3–5]. The microarray data have been deposited in NCBI's Gene Expression Omnibus (GEO) and can be accessed through GEO Series accession numbers GSE62062 and GSE63215.

Specifications	
Organism/cell line/tissue	*Homo sapiens*/H1/human embryonic stem cells
Sex	Male
Sequencer or array type	Illumina HumanHT-12 V4.0 expression beadchip (microarray)
Data format	Raw and analyzed
Experimental factors	hESCs were incubated in either hESC medium or hESC medium without bFGF and TGFβ for 0, 48 and 96 h. hESCs were treated with either DMSO, Aphidicolin or Aphidicolin + AZD7762 or with either control or CCNB1 overexpression lentiviral constructs.
Experimental features	Analysis of the effect of S phase arrest and replication checkpoint activation or Cyclin B1 overexpression on differentiating hESCs at the gene expression level.
Consent	N/A
Sample source location	Singapore

1. Direct link to deposited data

Deposited data can be found at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62062 and http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63215.

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2. Experimental design, materials and methods

2.1. Cell culture

The hESC line H1 (passage 30) was used for this study. They were cultured feeder-free on Matrigel (BD). hESCs were maintained by daily replenishment with mTeSR1 medium (STEMCELL Technologies). Routine subculture as clumps was executed when hESCs reach confluency every 4–5 days using 1 U/mL Dispase in DMEM/F12 (STEMCELL Technologies).

Prior to all treatments, hESCs were instead passaged as single cells using TrypLE™ Express (Life Technologies). 2 h after passage, treatments were applied. 24 h after treatment, medium was replaced to mTeSR1 without select growth factors (STEMCELL Technologies) to induce PSD (Fig. 1).

2.2. Experimental groups and conditions

To understand the effects of DNA replication perturbation and activation of the DNA damage checkpoint in hESCs undergoing PSD, we supplemented hESCs either with DMSO, with the DNA polymerase inhibitor Aphidicolin (75 ng/mL, Sigma), or with Aphidicolin plus the checkpoint kinase inhibitor AZD7762 (100 nM, SelleckChem).

To understand effects of Cyclin B1 overexpression in hESCs undergoing PSD, we infected hESCs either with the unmodified pLVTH vector or with the open reading frame of CCNB1 inserted using the PmeI and NdeI

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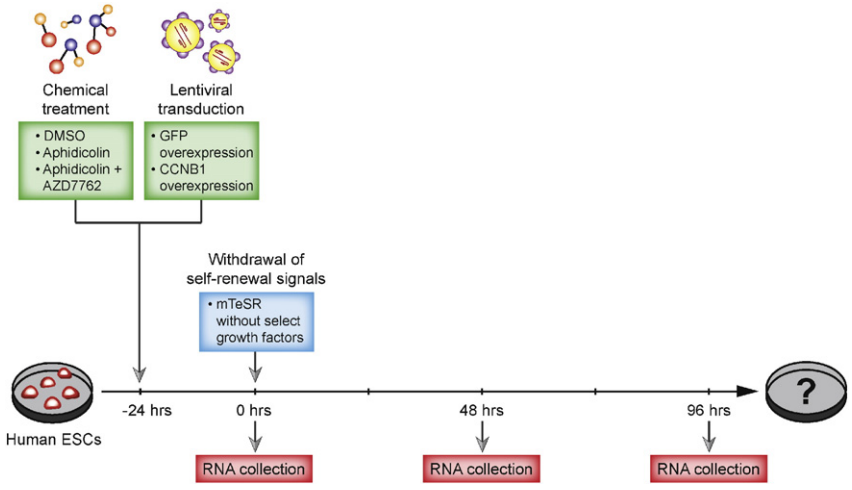

Fig. 1. Outline of time-course experiment prior to microarray analysis.

restriction sites. Lentiviral transduction was performed by addition of 2 μL of concentrated viruses to the culture medium with 4 μg/mL polybrene (Sigma).

Triplicate samples were prepared for each condition, and RNA was collected at 0, 48 and 96 h after induction of PSD (Fig. 1).

2.3. RNA extraction

Cells were lysed with TRIzol® (Invitrogen) and RNA was extracted using standard chloroform-isopropanol procedure. RNAs were purified by DNAse I treatment (NEB) followed by column-purification using PureLink RNA Mini Kit (Ambion).

2.4. Microarray hybridization and analysis

250 ng of purified RNA was used to synthesize cDNA, followed by *in vitro* transcription for 14 h and cRNA purification, all using Illumina TotalPrep RNA Amplification Kit (Ambion). 750 ng of the purified biotin-labeled cRNA was used for hybridization for 18 h onto the HumanHT-12 v4 Expression BeadChips.

After hybridization, the raw images were obtained using Illumina BeadArray Reader. Rank invariant normalization and analysis were performed using GenomeStudio Software. Only probes with detection p-value < 0.05 in all three replicates of at least one sample were considered for analysis. Differentially expressed genes were obtained using the Significance Analysis of Microarrays software (http://statweb.stanford.edu/~tibs/SAM/) and gene ontology analysis was done using DAVID (https://david.ncifcrf.gov/).

3. Discussion

Dramatic biochemical and physical changes occur during progression through the cell cycle. Cells have evolved to utilize the differences between cell cycle phases to prime or regulate other processes that are not directly related to proliferation [6–9]. We have demonstrated that interfering with DNA replication during the S phase or delaying progression in the G2 phase dominantly delays PSD [2]. Particularly, we find that the ATM/ATR-CHEK2-mediated DNA damage checkpoint and Cyclin B1 actively promote the pluripotent state in the S and G2 phases, respectively. To obtain a mechanistic connection between these pathways and pluripotency, we performed a time-course microarray analysis as described here. The datasets provided important information on the response of hESCs to DNA damage checkpoint activation, such as gene expression changes that lead to enhanced TGFβ signaling, which subsequently promotes the pluripotent state [3–5]. Similar changes were observed for Cyclin B1 overexpression, together with corresponding changes in mesoderm- and neuroectoderm-associated genes. In summary, our results demonstrate that the S and G2 phases possess an intrinsic propensity towards the pluripotent state, through active pathways that heighten TGFβ signaling. Knowledge on the regulatory network of PSD can be utilized to improve current differentiation protocols, and we hope that this resource will serve as a valuable tool for understanding PSD in hESCs and how this process is hardwired to the cell cycle.

Acknowledgments

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References

[1] C. Hindley, A. Philpott, The cell cycle and pluripotency. Biochem. J. 451 (2) (2013) 135–143.
[2] K.A. Gonzales, et al., Deterministic restriction on pluripotent state dissolution by cell-cycle pathways. Cell 162 (3) (2015) 564–579.
[3] R.H. Xu, et al., NANOG is a direct target of TGFbeta/activin-mediated SMAD signaling in human ESCs. Cell Stem Cell 3 (2) (2008) 196–206.
[4] D. James, et al., TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. Development 132 (6) (2005) 1273–1282.
[5] L. Vallier, et al., Activin/Nodal signalling maintains pluripotency by controlling Nanog expression. Development 136 (8) (2009) 1339–1349.
[6] K. Handschick, et al., Cyclin-dependent kinase 6 is a chromatin-bound cofactor for NF-kappaB-dependent gene expression. Mol. Cell 53 (2) (2014) 193–208.
[7] Y. Lee, et al., Cyclin D1-Cdk4 controls glucose metabolism independently of cell cycle progression. Nature 510 (7506) (2014) 547–551.
[8] S. Pauklin, L. Vallier, The cell-cycle state of stem cells determines cell fate propensity. Cell 155 (1) (2013) 135–147.
[9] F. Rodier, et al., Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat. Cell Biol. 11 (8) (2009) 973–979.