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FRONTIERS IN LIFE SCIENCE

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PCEI-HU <Photoswitchable CENP-E Inhibitor>

Catalog NO. FDV-0037

Product Background

Mitosis is a fundamental process of cell duplication for the growth and maintenance of tissues in multicellular organisms. During mitosis, many kinds of proteins play crucial roles in accurate distribution of the replicated genome into two daughter cells. The replicated genomes are condensed into chromosomes in the prophase and subsequently, chromosomes move to the center of the mitotic cells, frequently called metaphase plate, along spindle microtubules in prometaphase. Chromosomes bind to spindle microtubules via kinetochore which is a unique protein complex in centromere. Only when all chromosomes are aligned to the metaphase plate in metaphase, separation of sister chromosome begins (anaphase). There is an important checkpoint called spindle assembly checkpoint (SAC) from metaphase to anaphase. SAC monitors accurate alignment of chromosomes in metaphase plate in mitosis to exact separation of genomic information into daughter cells. Accurate segregation of all chromosomes is the most important matter to satisfy the SAC criteria. **Cen**tromere **p**rotein **E** (CENP-E) is a key component for chromosome congression along microtubule spindles to the metaphase plate. CENP-E is a dimeric protein which has three domains, N-terminal motor domain, C-terminal kinetochore binding domain and coiled-coiled linker region. Recent studies suggest inhibition of ATPase activity of the CENP-E motor domain suppresses chromosome congression to the metaphase plate, subsequently SAC detects the misalignment of chromosomes and as a result, anaphase does not start. Controlling CENP-E activity has a large potential to control mitosis progression. Although some potent CENP-E inhibitors have been developed so far, these inhibitors are not suitable for reversibly controlling CENP-E activity.

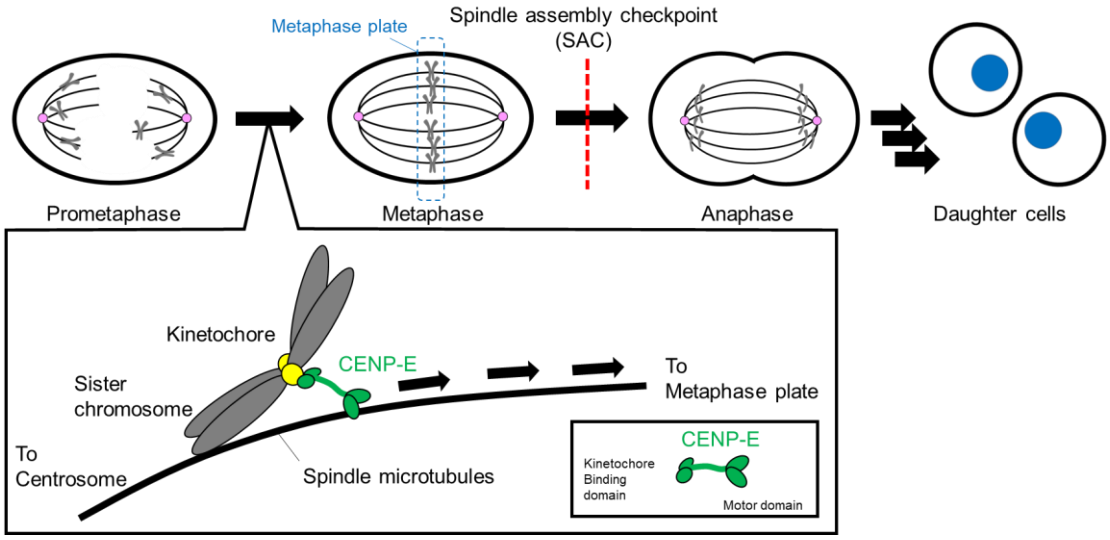


Figure 1 Overview of CENP-E in mitosis

Dr. Tamaoki, Dr. Uehara and co-workers, Hokkaido University, succeeded in obtaining the world's first **p**hotoswitchable **C**ENP-**E** **i**nhibitor (PCEI-HU) which contains an azobenzene-based photo-isomerization unit. PCEI-HU can regulate CENP-E activity ON and OFF repeatedly by UV and visible light (Vis) irradiation. PCEI-HU in water without light shows potent CENP-E inhibitory activity, but once UV light irradiation, PCEI-HU lacks its inhibitory activity. Under the condition, CENP-E keeps its motor activity for chromosome congression to the metaphase plate. This UV-induced loss of inhibitor action is recovered by Vis light. After Vis irradiation, PCEI-HU blocks CENP-E activity again and chromatin congression is clearly suppressed. PCEI-HU is a powerful tool for the fundamental investigation of prometaphase/metaphase and SAC.

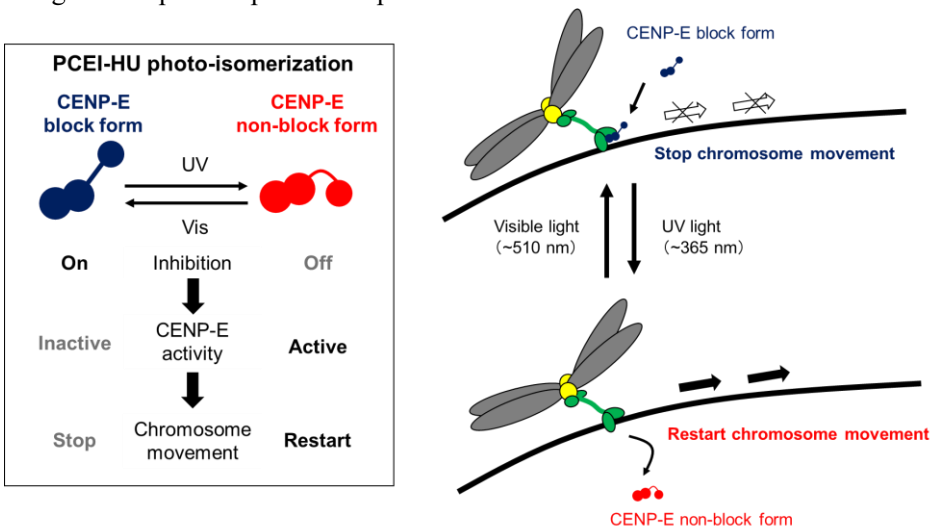


Figure 2. Principle and overview of PCEI-HU

Description

Catalog Number: FDV-0037
Size : 50 µg
Formulation : $C_{26}H_{31}N_6O_3Cl$
Molecular weight : 511.02 g/mol
Solubility : Soluble in DMSO

Application

- Photoswitchable CENP-E inhibitor
- Photoswitchable chromosome movement in prometaphase/metaphase during mitosis

Reconstitution and Storage

Reconstitution : Add 100% DMSO into vial to prepare 1-10 mM stock solution.
Storage :
Powder : Store at -20 °C with light shedding.
Stock solution :
- Make aliquots and store at -20 °C with protection from light.
- Avoid repeated freeze-thaw cycles.
- To avoid photo-induced isomerization of PCEI-HU, one-time use of each aliquot is highly recommended.

<NOTE> PCEI-HU may be isomerized by environmental light such as room light or sunlight. When making and storing aliquots, please carefully check the light conditions around the experimental space.

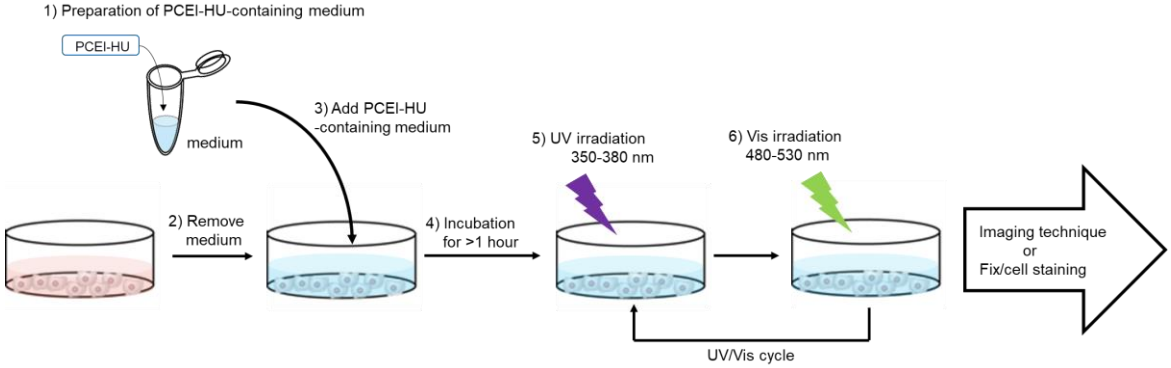
How to use

Cell-based CENP-E inhibition assay

1. Add PCEI-HU (for example, final. 1 μM for HeLa cells) in fresh media in the dark condition.
2. Remove the cultured medium and wash cells by PBS several times.
3. Add PCEI-HU containing medium to the cells.
4. Culture cells for 1-2 hours in dark condition
5. UV-irradiate culture dish by any light sources.
6. Vis-irradiate culture dish by any light sources.

*NOTE(1): Photo-irradiation condition should be optimized by users for each experiments. Detail information is described in the later chapter.

*NOTE(2): All procedures are recommended in the dark condition to avoid non-specific photoisomerization of PCEI-HU by environmental light source.

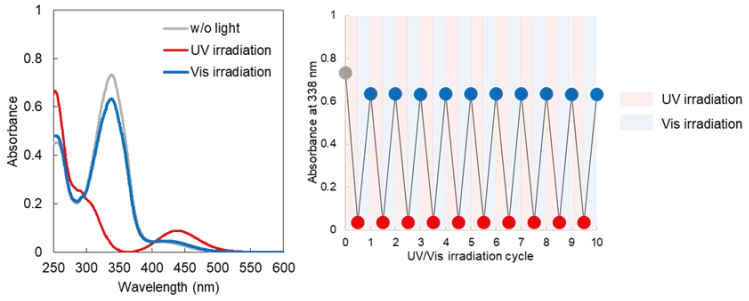


Reference data

Absorption spectrum

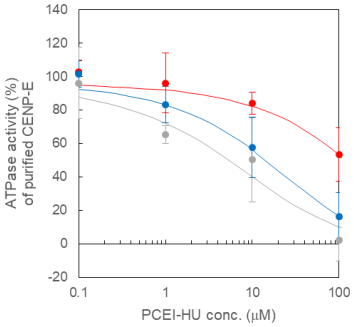
Left: Absorption spectrum of PCEI-HU. Without light irradiation (w/o light; gray), after 365 nm light irradiation (UV irradiation; red) and after 510 nm light irradiation (Vis irradiation; blue).

Right : Absorbance changes at 338 nm under alternating 365/510 nm illumination.



Inhibition activity for purified CENP-E ATPase of PCEI-HU

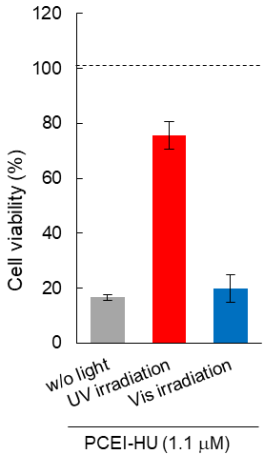
ATPase activities of CENP-E with various concentrations of PCEI-HU were measured. IC_{50} values indicate, inhibition activity of PCEI-HU after UV irradiation was about ten-fold decreased than that after Vis irradiation.



Condition	IC_{50}
w/o light	5.9 μM
UV irradiation	120 μM
Vis irradiation	14 μM

Cytotoxicity of PCEI-HU for long-term culture

HeLa cells were treated with 1.1 μM PCEI-HU under three conditions, w/o light, after UV irradiation and after Vis irradiation for 42 hours. W/o light and Vis irradiation show potent cytotoxicity. On the other hand, UV irradiation keeps high cell viability. Treatment of 1 μM PCEI-HU, a recommended concentration, for a few hours, has little effect on cell viability.



Experimental guides

Light source of photoirradiation

For UV irradiation, 350-380 nm light is recommended. In the case of 365 nm light, 33 mW/cm^2 for 25 sec is enough to convert PCEI-HU.

For Vis irradiation, 480-530 nm light is recommended. In the case of 510 nm light, 117 mW/cm^2 for 35 sec is enough to convert PCEI-HU.

In this manual, all data were obtained using 365 nm light-emitting diode (LED, C11924-101, Hamamatsu) and 510 nm LED (CS-LED3W_510 nm, Hayasaka).

Photoisomerization ratio of PCEI-HU

“CENP-E block form” and “CENP-E non-block form” were in photoisomerization equilibrium. Under the adequate irradiation of UV (365 nm) light, the ratio of “CENP-E block form”/”CENP-E non-block form” was 7/93%. Under the adequate irradiation of Vis (510 nm) light converts 86/14%. Please note both UV and Vis light irradiation did not convert to “CENP-E block form” and “CENP-E non-block form” completely.

Note for live cell imaging

As PCEI-HU is a photo-responsive reagent, excitation light for any fluorophores (fluorescent dyes, proteins, etc.) should be carefully selected under live-cell imaging. Ideally near-infrared fluorophores excited by over 630 nm light are compatible with PCEI-HU without any effect on PCEI-HU photoisomerization.

Validated species

PCEI-HU was only validated in human cell lines and human CENP-E protein. There is no data for other species such as mice, rat, etc.

Controllable capacity

Ref.1 revealed that once chromosomes reached to the metaphase plate, PCEI-HU cannot regulate further chromosome movement. Please carefully check the status of chromosome alignment. (See Application data).

Application data

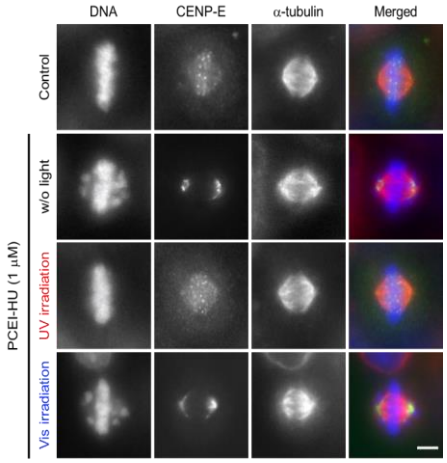
Cellular localization of chromosomes, CENP-E and tubulin under photo-dependent PCEI-HU

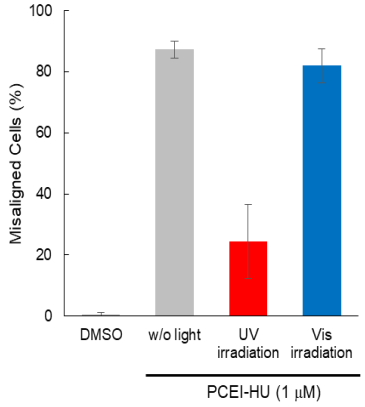
HeLa cells were treated with 1 μM PCEI-HU and 20 μM MG132[*1] under following three conditions. After these conditions, cells were fixed with PFA and visualized by specific antibodies for CENP-E and α-tubulin and DAPI staining for chromosomes.

1) Add PCEI-HU without light for 2 hours → fixation = "w/o light"
2) Add PCEI-HU without light for 2 hours → after UV irradiation, culture for 30 min = "UV irradiation"
3) Add PCEI-HU without light for 2 hours → after UV irradiation, culture for 5 min
   → after Vis irradiation, culture for 30 min = "Vis irradiation"

Under the condition 1), PCEI-HU w/o light works as the "CENP-E block form" and induced misalignment of chromosomes. CENP-E was highly accumulated in the centrosome. Under the condition 2), UV irradiation converted PCEI-HU to "CENP-E non-block form" and all chromosomes reached to the metaphase plate. CENP-E was distributed near metaphase plate. Under condition 3), Vis irradiation recovered CENP-E inhibitory activity of PCEI-HU and shows a similar result with 1) w/o light.

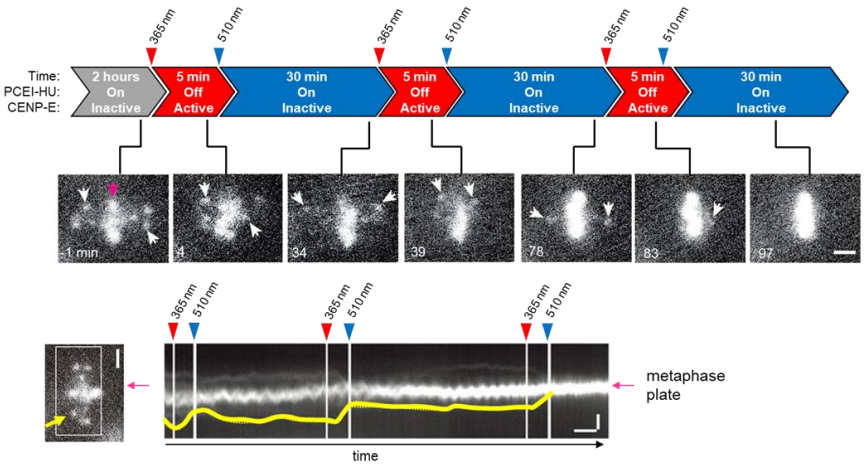
NOTE *1: MG132 is used as an anaphase inhibitor. Under MG132 treatment, mitosis did not enter anaphase even when all chromosomes were aligned in the metaphase plate.





Live cell imaging of mitotic chromosomes in PCEI-HU treated LLC-PK 1 cells

LLC-PK1 cells were treated with near-infrared DNA staining dye (SiR-DNA, 1 μM) and subsequently 1 μM PCEI-HU and 20 μM MG132 for 2 hours in darkroom. Cells were irradiated with UV light (365 nm) and Vis (510 nm) repeatedly and monitored in live-cell (Upper figure). Movement of a specific chromosome (yellow arrow) was analyzed in kymograph (Lower figure). While after UV irradiation, chromosomes moved to metaphase plate, but after Vis irradiation chromosomes left from metaphase plate. The chromosome was repeatedly regulated by UV/Vis cycles until chromosomes reaching to the metaphase plate.

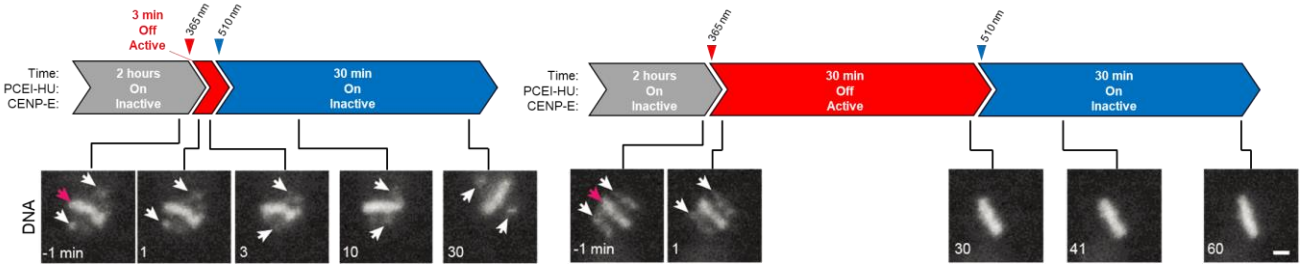


Elucidation of CENP-E roles after chromosome congression to the metaphase plate

HeLa cells were treated with 1 μM PCEI-HU and 20 μM MG132 under the following two conditions.

1) Add PCEI-HU without light for 2 hours → after UV irradiation, culture for 3 min → after Vis irradiation, culture for 30 min (Left figure)
2) Add PCEI-HU without light for 2 hours → after UV irradiation, culture for 30 min → after Vis irradiation, culture for 30 min (Right figure)

When the incubation time after UV irradiation was short such as 3 min, Vis irradiation recovered the suppression of chromosome movement. However, if the incubation time after UV irradiation was long such as 30 min, chromosomes reached to metaphase plate and could not be controlled by Vis irradiation again. This experiment showed once chromosomes reached to the metaphase plate, PCEI-HU could not regulate further chromosome movement.

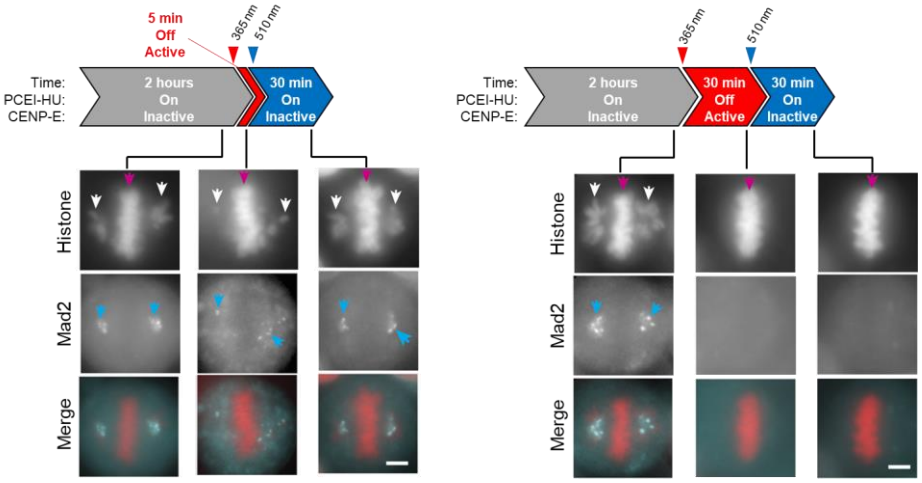


Functional assay to monitor a movement of Mad2, a SAC-regulatory protein

HeLa cells co-expressing Mad2-GFP and histone H2B-mCherry were treated with PCEI-HU and MG132 under the following two conditions.

1) PCEI-HU w/o light for 2 hours →after UV irradiation, culture for 5 min →after Vis irradiation, culture for 30 min →fixed and observed
2) PCEI-HU w/o light for 2 hours →after UV irradiation, culture for 30 min →after Vis irradiation, culture for 30 min →fixed and observed

In both conditions, PCEI-HU w/o light-induced misalignment of chromosomes and aggregation of Mad2 in misaligned chromosomes. In condition 1), short-term culture after UV irradiation Mad2 remained aggregated in the misaligned chromosome. On the other hand, condition 2) showed long-term culture after UV irradiation induces correct alignment of chromosomes and Mad2 was clearly diffused in the cytosol. These results indicate Mad2 specifically accumulated in misaligned chromosomes and after diffused in the cell by a complete alignment of chromosome in the metaphase plate.



Reference

1. Mafy, N. N. *et al., J. Am. Chem. Soc.*, **142**, 1763-1767 (2020) Photoswitchable CENP-E Inhibitor Enabling the Dynamic Control Chromosome Movement and Mitotic Progression.

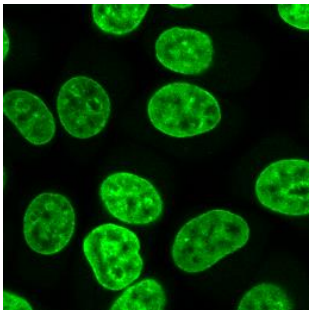
Related products:

NucleoSeeing <Live Nucleus Green>

NucleoSeeing is DNA-responsive green dye for monitoring cell nucleus in live cells. As it shows low cytotoxicity and phototoxicity, it is very suitable for long-term live imaging of cell nucleus.

Catalog No. FDV-0029
Size 0.1 mg
Features

- Easy and quick procedure
- Compatible with 10% FBS
- Validated for both adherent cells and floating cells
- Little influence on cellular functions
- Ex/Em: 488 nm/520 nm (commercial FITC filters are available)



NOTE: NucleoSeeing is not compatible with PCEI-HU experiments. For PCEI-HU experiments in live cells, please use near-infrared (NIR) dyes.

Gatastatin G2 <γ-Tubulin Inhibitor>

Gatastatin G2 is a second generation of γ-tubulin specific inhibitor, Gatastatin. Gatastatin G2 has little effects on α/β–tubulin polymerization both *in vitro* and in cells and induced abnormal chromosome alignment and multipolar formation in mitotic cells.

Catalog No. FDV-0040
Size 0.1 mg
Features

- γ-tubulin specific inhibitor
- blocks GTP-binding of γ-tubulin
- useful for functional analysis of multifunction of γ-tubulin

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