CEPT supplementation demonstrates significant advancement in survival of genetically stable hPSCs

Summary

Human pluripotent stem cells (hPSCs) have extensive self-renewal capacity in culture, yet are highly sensitive to their in vitro environment, especially when cells undergo stressful culture manipulations, such as dissociation as single cells during routine passaging or clonal culture. Stressful culture processes such as these often yield poor cell survival. Clinical translation of hPSCs requires well-controlled, safe, and efficient strategies for cell culture expansion and large-scale production, clonal manipulation, cryopreservation, and directed differentiation.

An innovative and targeted high-throughput screen and assessment of compounds has found that supplementation with CEPT, the combination of Chroman 1, Emricasan, a Polyamine solution, and Trans-ISRIB, can be safely used in research, regenerative medicine, cryobiology, and drug development applications to improve the viability and expansion of hPSCs in culture, as well as improve functionality of multiple derived cell types.

The Spotlight

Research area: Pluripotent stem cell culture, long-term culture, single-cell dissociation, clonal cell expansion, iPSC reprogramming

Cell type(s): hPSCs, hiPSCs, hESCs

Experiment purpose: A targeted high-throughput analysis to identify and characterize a synergistic combination of small molecules and culture additives that can be safely used in research and regenerative medicine applications to improve the viability and expansion of hPSCs in culture.

Product Highlights

CET Cocktail

The Captivate Bio CET Cocktail is comprised of the small molecule components in the CEPT cocktail: Chroman 1, Emricasan, and Trans-IRIB (CET).

- Enhances stem cell survival and expansion
- Promotes clonal growth of genetically stable hPSCs
- Provides comprehensive cytoprotection

Experiment overview

Targeted high-throughput compound screening

- Chroman 1 identified as a potent ROCK inhibitor, generating cell viability results superior to Y27632.
- Chroman 1 is more selective than Y27632, with no off-target effects.
- Long-term culture with Chroman 1 at each passage yielded genetically stable, pluripotent hPSCs.

Screening to further enhance cell survival

- Emricasan, a pan-caspase inhibitor, was found to enhance cell survival synergistically with Chroman 1.
- Combining Emricasan and Chroman 1 was the most efficient at minimizing cell death following single-cell dissociation, and maintained healthy, pluripotent hPSCs for over 40 passages in culture.

Analysis of cell survival under ultra-low cell density conditions

- Trans-ISRIB and polyamines further improves cell culture when combined with Chroman 1 and Emricasan supplementation.
- Termed CEPT cocktail, this combination of small molecules effectively promotes cell attachment, survival, and protein synthesis.

Validation of long-term culture with CEPT cocktail

- Eight hPSC lines were cultured for routine long-term passaging with the CEPT cocktail (exposed to cells 24 hours at each passage) prior to a detailed analysis to show expected morphology, high pluripotency, and normal karyotypes.
- Long-term passaging in multiple systems demonstrated that the improved cell survival effects of CEPT are independent of the cell line, media, or culture substrate.

Results

Chroman 1 is a potent and selective ROCK1/2 inhibitor. A high-throughput screen focusing on cell viability was performed to identify novel chemical compounds that effectively promoted cell survival. A total of 15,333 compounds were screened on 1536-well plates using diverse small molecule libraries. From these screening assays, the small molecule Chroman 1 stood out as the key compound that improved cell viability.

Chroman 1 is a highly potent and selective ROCK inhibitor, found to be more potent than Y27632, the most widely-used ROCK inhibitor for stem cell cultures today. Chroman 1 generated similar improvement in cell viability used at only 50 nM as Y27632 when used in the standard application of 10 μM. Additionally, Chroman 1 was found to be more specific than Y27632, showing no inhibition of off-target kinases at 50 nM.

Importantly, Chroman 1 was shown to be a safe supplement in long-term cell culture. Cells cultured with Chroman 1 for the first 24 hours of each passage via single-cell dissociation remained karyotypically normal after 40 passages with the treatment. In addition, the cultures showed typical morphology and demonstrated expression of pluripotency markers, as well as maintained the ability to differentiate into endodermal, mesodermal, and ectodermal lineages.

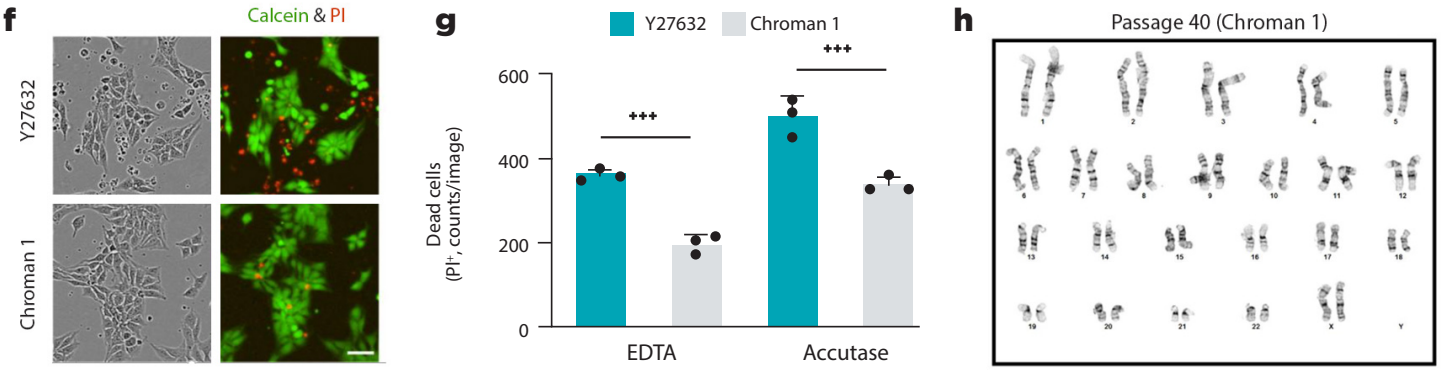

Figure 1. Cell morphology and survival. Improved cell survival after single cell dissociation by either EDTA or Accutase and low-density seeding (at 100,000 cells/cm^2) was observed in hPSC cultures treated with 50 nM Chroman 1 in the culture media for the first 24 hours post-seeding (H9 hESC line, shown in data) as seen by phase contrast and fluorescent images, as well as live/dead cell counts taken 12 hours after cell plating (f, g). Cells cultured with 50 nM Chroman 1 for the first 24 hours of each passage via single-cell dissociation remained karyotypically normal after 40 passages with the treatment (h). Please see the publication for a complete review of the data shown, published from Yu Chen et al. (2021) Nature Methods.

Chroman 1 and Emricasan work in synergy to improve cell survival.
A combinatorial matrix screening approach was used to identify any synergistic compounds that further improved cell survival with Chroman 1 application. Emricasan was identified as the pan-caspase inhibitor that proved to be most effective in combination with Chroman 1. The combination of Chroman 1 and Emricasan was found to be superior and improving cell survival than using either Chroman 1 or Y27632 alone.

After single-cell dissociation, a significant increase in the number of viable cells was observed when cultures were treated with Chroman 1 and Emricasan. Cells remained karyotypically normal when dissociated as single cells for 40 passages and treated with this small molecule combination during each passage.

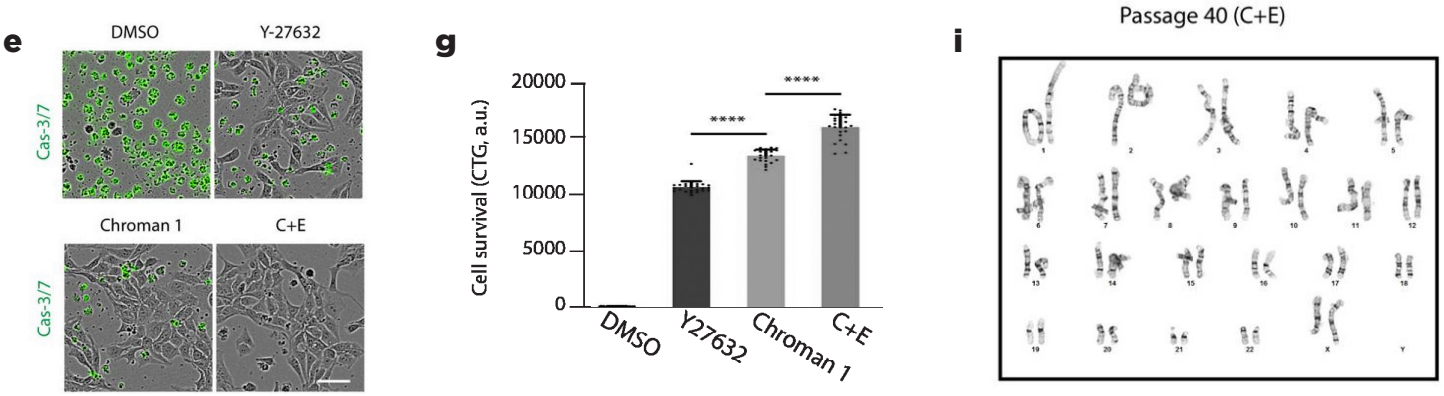

Figure 2. Small molecules enhance cell survival. When the small molecule combination of Chroman 1 (50 nM) and Emricasan (5 μM) was applied to hPSCs for the first 24 hours after single-cell dissociation, improved cells survival and a decreased number of apoptotic cells were seen, observed by a clear reduction in caspase activity (e) as well as increased cell viability (g). Cells passaged with both Chroman 1 and Emricasan at each passage were karyotypically normal after 40 passages of serial treatment (i). Published data from Yu Chen et al. (2021) Nature Methods.

Supplementation allows for safe and efficient cell passaging and clonal expansion from low densities.
Additional screening identified compounds that would further enhance the effects of Chroman 1 and Emricasan under extreme conditions, such as ultra-low cell densities. Trans-ISRIB, a selective small molecule integrated stress response (ISR) inhibitor with a key role in regulating protein translation, showed the most significant improvement to cell survival when used in combination with Chroman 1 and Emricasan.

Further experiments showed that supplementing polyamines with Trans-ISRIB compound promoted cell attachment and protein synthesis during cell passaging when combined with Chroman 1 and Emricasan. The combination of Chroman 1 (50 nM), Emricasan (5 μM), and Trans-ISRIB (0.7 μM) small molecules in culture media additionally supplemented with Polyamine Solution promotes clonal growth and expansion of genetically stable hPSCs.

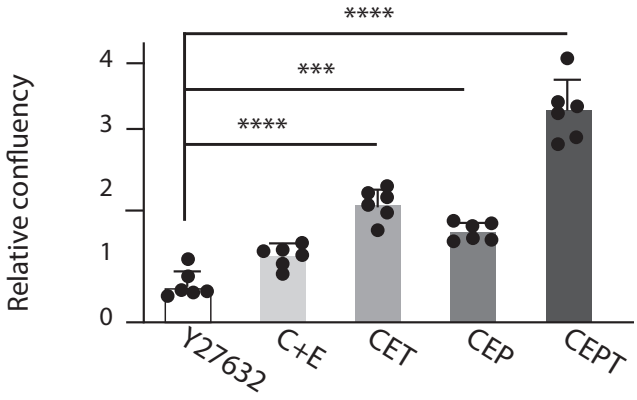

Figure 3. Cell survival. hPSCs were seeded as single cells at an ultra-low density of 25 cells/cm^2 and incubated with Y27632 ROCK inhibitor; Chroman 1 and Emricasan (C+E); Chroman, Emricasan, and Trans-ISRIB (CET); Chroman 1, Emricasan, and polyamines (CEP); and complete CEPT cocktail (Chroman 1, Emricasan, Trans-ISRIB, and polyamines); highlighting combinatorial improvements to cell survival (b). Published data from Yu Chen et al. (2021) Nature Methods.

Long-term passaging with exposure to CEPT maintains healthy, pluripotent cultures.
Eight hPSC lines were cultured with CEPT (exposed to cells 24 hours at each passage) prior to a detailed analysis. Throughout this long-term study, it was seen through the vast majority of the cells remained undifferentiated. The cells retained normal karyotypes and were capable of directed and spontaneous differentiation. No changes were observed by whole exome sequencing and genetic analysis when cells were passaged with CEPT for 20 passages.

Cells were transferred to a variety of culture conditions to demonstrate that the improved cell survival effects of CEPT are independent of the cell line (hESC or hiPSC), media composition (E8, StemFlex®, mTeSR®, StemFit®, MEF-conditioned medium), or coating substrate (vitronectin, Matrigel®, laminin-521).

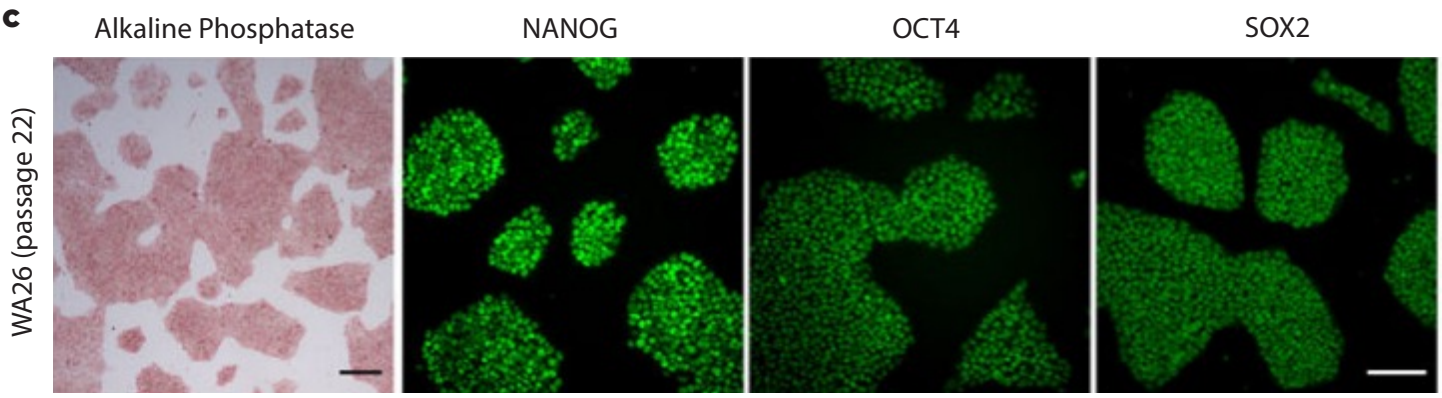

Figure 4. Pluripotentcy marker expression. Representative images showing that hPSCs colonies express typical pluripotency-associated markers (Alkaline Phosphatase, NANOG, OCT4, SOX2) after long-term serial passaging with CEPT (c). Scale bar, 200 μm. Published data from Yu Chen et al. (2021) Nature Methods.

Conclusion

The synergistic effects of the CEPT cocktail allow for safe and efficient stem cell passaging and clonal expansion from low densities. Control over viability, safety, and cell function are key challenges facing translational stem cell research. There is currently no consensus in the field on the best method for routine passaging of hPSCs, and labs often overcome poor cell survival using a variety of compensation methods. Supplementation with CEPT provides chemical protection as a new approach for more standardized and efficient hPSC culture.

References

1. Yu Chen, et al. (2021) A Versatile Polypharmacology Platform Promotes Cytoprotection and Viability of Human Pluripotent and Differentiated Cells. Nature Methods. May; 18(5): 528-514.

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