Adverse Effects of Culture Media on Human Pluripotent Stem Cells

Supplemental experimental procedure

Quantification of nuclear size and shape: Cellprofiler (Carpenter et al., 2006) was used to identify and measure nuclei of HPSCs under different conditions. Nuclear volume was calculated as the product of average nuclear area and the average depth of the nuclei in the image stacks which encompassed the entire nucleus.

Quantification of nuclei with different number and type of nucleoli: The 'cell counter' plugin was used on ImageJ to mark nuclei with different numbers of nucleoli and finally expressed as percentage of the total number of nuclei counted. These analyses were blinded.

Immunostaining: Cells were fixed with 4% paraformaldehyde followed by permeabilization with 0.01% Triton-X.1% BSA was used for blocking. Incubation with primary antibodies was at 4^0C overnight or at room temperature for 1 hour. For secondary antibodies, incubation time was 1 hour at room temperature.

Quantification of 8-hydroxyguanosine, phospho-Histone H2A.X and p53 immunostaining: For each of the above experiments, at least five images were acquired per condition per biological sample as image stacks. Fluorescence intensity was calculated per nuclei (equivalent to fluorescence intensity/cell as in FACS analyses) by multiplying the fluorescence intensity per nuclear area by the average depth of the nuclei.

For quantification of fold change in DSBs after irradiation, cells were irradiated with 5 Gy and placed in the incubator for 1 hour after which cells were fixed along with untreated cells. Values were expressed as fluorescence intensity per nuclei of irradiated cells over untreated cells.

Quantification of percentage of normal and aberrant mitotic figures: Normal mitotic figures were identified based on Hoechst staining of nuclei which showed chromosomes in different mitotic phases as described in an earlier study (Momcilovic et al., 2010). Aberrant mitotic figures were identified based on Hoechst staining of nuclei showing trailing DNA segments as described in a recent study (Golan-lev et al., 2016) as well as those which showed severely mis-arranged chromosomes (as represented in figure S2B and S2C). Both were expressed as a percentage of total nuclei counted (>1500).

γ-irradiation and doxorubicin treatment: Cells were irradiated with 5 Gy after which they were placed at 37^0C and 5% CO_2 for an hour. Doxorubicin was added at 1μM concentration to cells and incubated at 37^0C and 5% CO_2 for three hours. After both these treatments, cells with the respective untreated controls were fixed with 4% paraformaldehyde and imaged directly or processed further for immunocytochemistry experiments.

Supplemental figures

Figure S1 related to figure 3

HPSCs in E8 and mTeSR media show higher nuclei acid damage when compared to that in KSR media.

(A) Representative images showing higher 8-OH guanosine immunostaining of HPSCs in mTeSR media followed by E8 media and negligible levels in KSR media. Nuclei are counter-stained with Hoechst 33342. There is a prominent nucleolar marking by 8-OHG antibody, especially in mTeSR media. (B) Representative wide-field microscopy and (C) confocal images of Hoechst staining of HPSCs in E8 & mTeSR and E8 media only showing aberrant mitotic figures. Scale bars represent 10µ.

Figure S2

Nucleolar morphology can act as *"stress reporter"* in HPSCs

Representative (A) confocal images of Hoechst staining and (B) bright field images of HPSCs cultured in KSR media and treated with genotoxic stress – i.e., irradiation (irr) and doxorubicin (DR) showing clear, distinct and rounded nucleoli in KSR DR followed by mTeSR while reticulate and less defined nucleoli in KSR only. (C) Quantification of percentage of clear and distinct nucleoli under above mentioned conditions; n=6. Scale bars represent 10µ. Pooled data from all the four cell lines and represented as mean ± SEM. Student's t-test. *** $p<0.001$, * $p<0.05$.

Supplemental references

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