Should Human Induced Pluripotent Stem Cells Regret their Choice of DNA Repair Mechanisms?

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Human induced pluripotent stem cells (hiPSC) have a huge potential use in regenerative medicine. However, the mutant frequency of hiPSC reaches the level of differentiated cells rather than that of similar cell type, the human embryonic stem cells (hESC). hESC on the other side were shown to lose their genomic stability during the cultivation due to decrease of DNA base excision repair (BER) activity. It was shown that the lack of BER results in replication stress in hESC. I have thus focused my attention on studying the replication stress and its relation to BER activity level in hiPSC using analysis of replications forks. The presence of stalled forks in unirradiated hiPSC and subsequent accumulation of late replication origins suggests higher level of intrinsic replication stress in hiPSC. Further low sensitivity of replication speed to BER inhibition in presence of oxidative DNA damage suggests low BER activity in hiPSC. I further focused on analysis of processing of the DNA damage induced by replication stress, especially if it is compensated for by elevated activity of HR. I analyzed the level of ?H2AX and RAD51 foci. Presence of RAD51 foci, which do not colocalize with ?H2AX foci, suggests high level of replication stress. Further, the high level of ?H2AX foci, which do not colocalize with RAD51 suggests large portion of DNA damage not being processed via HR and thus contribute to the genomic instability of hiPSC. I conclude that preparation of hiPSC with stable genome requires modulation of DNA damage response in hiPSC.

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