Oxidative stress plays a role in the development as well as the degeneration of the brain. Oxygen levels in the developing embryo are low and proper regulation of oxygen levels is crucial for stem cells, neural progenitor cells and neurons. In vivo models enable examination of the mechanisms involved in neural development, neuronal function and neuronal responses to injury. However, most neuronal culture methods employ non-physiological oxygen conditions. Standard cell culture is performed at atmospheric (21%) oxygen levels. The ability to study oxidative stress in neurons in hyperoxic (atmospheric) conditions is particularly problematic when studying disorders that are characterized by oxidative stress. Therefore, it is crucial to investigate oxidative stress features of a context in which every alteration is oxygen dependent. Recently, it has become clear that cultivating cells in particular stem and neural progenitor cells, in low (physiological, 3.5%) oxygen makes the cells grow faster, healthier, less stressed, and with less DNA damage. We therefore investigated the role of oxidative stress in human induced pluripotent stem cells (iPSC) cells under varying oxygen levels. Expression of several genes involved in the cellular oxidative stress response when cells were maintained in different oxygen concentrations. iPSC cells were cultured in 5% oxygen (physiological) or 21% oxygen (atmospheric) and analyzed for expression of specific genes known to be involved in oxidative stress (SODD, APP, DSCR, ETA, SI010).

EXPERIMENTAL DESIGN

We differentiated them into forebrain neurons. While these human DS neurons appear to be less regulated and changes can have profound effects on function (Hoge and Pike, 2007).

METHODS

To test whether human pluripotent stem cells show oxidative stress responses in low oxygen, cells were cultured in both atmospheric and physiological oxygen levels. The expression of specific genes involved in oxidative stress was measured.

RESULTS

Gene expression: HS21 genes (SODD, APP, ETA2, SI010) and non-HSA 21 genes (catalase, zeta-crystallin) were evaluated and compared between cells cultured in both atmospheric and physiological oxygen conditions.

Quantitative PCR was performed using the automated pipetting workstation PIPETMAX™ qPCR Assay in order to maintain manual pipetting influences. Plate setup enabled transition to VIA thermocycler. The results show that the expression of all of these genes is reduced when iPSCs are cultured in low oxygen. These results have implications for culturing and analyzing disease specific iPSC to model diseases that have an oxidative stress component.

CONCLUSIONS

The expression of oxidative stress genes was generally increased in trisomy 21 iPSC compared to controls, corroborating our previous data (Weick et al., 2013). Control high vs Trisomy 21 high. Trisomy 21 cells grown in low oxygen have decreased expression of many oxidative stress genes. Most genes are over expressed in Isogenic trisomy 21 iPSCs (SOD). The expression of oxidative stress genes is affected by low oxygen conditions. Maintenance of cells in low oxygen cause decreased expression of many oxidative stress genes in Trisomy 21 iPSCs.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Jerome Lejeune Foundation, and in part by a core grant from the NICHD (P30 HD033532).