Julia Sprenger
REPR_SCI_455
2/4/2024

Descriptive Writing Assignment: Ciampa et al. (2023)

Figure 2. *To validate the dynamic pathway activity suggested by the WGNCA…..*qPCR assessed the expression of mRNA of *Glb1*, a senescence marker, between embryonic days 13.5, 15.5, and 17.5 of development. The results align with the WGCNA data, showing that cell senescence in the placenta is at its highest on e17.5, towards the end of gestation ($p = 0.0048$, Figure 2B). A western blot analysis was employed to quantify the abundance of hypoxia-inducible cofactor (HIF-1) at different stages of embryonic development in comparison to actin, a loading control. On e17.5, HIF-1 was found to be significantly greater than on e13.5 or e15.5 ($p = 0.019$, Figure 2C). qPCR corroborated these findings by analyzing the regulation of two targets of HIF-1: Hk2, and Slc2a1. Both factors were significantly upregulated on e17.5 compared to e13.5 and e15.5, supporting that HIF-1 is upregulated as gestational age increases ($p < 0.0001$, Figure 2D). Western blot analysis revealed that the expression of COX IV, a protein marker for mitochondrial abundance, decreased with gestational age, suggesting mitochondrial dysregulation ($p=0.0064$, Figure 2E). These results indicate that mouse placental aging, across a healthy pregnancy, is characterized by cellular senescence, HIF-1 signaling, and mitochondrial dysregulation.

Figure 4. Using a western blot analysis, there was a notable accumulation of HIF-1 protein in cultured trophoblasts exposed to CoCl2 for 6 hours (Figure 4A). Subsequently, following 48 hours of $CoCl_2$ exposure, there were significant reductions in the mRNA expression of *Cox2* and expression of COX IV ($p = 0.014$, Figure 4B and $p = 0.0047$, Figure 4C). These results suggest that exposure to CoCl2 causes a significant decrease in mitochondrial abundance. Lastly, the expression of senescence-associated beta-galactosidase (SA-βGal), encoded by Glb1, increased. This can be visually detected as a distinct blue stain in an X-gal assay designed to identify senescence ($p = 0.012$, Figure 4E). These findings collectively suggest that subjecting trophoblast cells to $CoCl_2$, thereby stabilizing HIF-1, induces a cascade of events leading to downstream mitochondrial dysfunction and eventual senescence of trophoblast cells.