Placental Exposure to Hypoxia and Oxidative Stress Causes Mitochondrial DNA Release into the Extracellular Space

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Background

In preeclampsia, a severe hypertensive disorder of pregnancy, placentae experience reduced perfusion, increased cell death, and oxidative stress, with an increase in circulating cell-free mitochondrial DNA (mtDNA). Our lab has shown that a synthetic mimetic of mtDNA during rodent pregnancy increases maternal blood pressure and vascular reactivity, features of human preeclampsia. The main objective of this study was to determine the role of hypoxia and oxidative stress in mtDNA release from placental cells, and to examine the effects of soluble factors from hypoxia-exposed placentae on vascular reactivity.

Hypotheses

a) Exposure to hypoxia and oxidative stress will result in mtDNA release via cell-death dependent mechanisms in human trophoblast cells.

b) Soluble factors from hypoxia-exposed placentae will result in reduced vasodilation in rat maternal arteries.

Methods

To examine the effects of preeclampsia-related placental stressors on mtDNA release, we treated human trophoblast cells (BeWo cell line) with: 1) hypoxia (1% O2) vs. normoxia (21% O2) for 15 h, or 2) a mitochondrial complex I inhibitor (Rotenone, 10 μM) vs. vehicle for 4 h. mtDNA in cell culture supernatant was measured using absolute qPCR and cell death was quantified using flow cytometry. To test the effects of hypoxic placenta-derived factors on maternal vascular function, we used mesenteric arteries and placenta-conditioned media (PL_media) from pregnant rats. Placentae were incubated in physiological salt solution (37°C) for 3 h in either 1% or 21% O2, while arteries were mounted on a wire myograph and underwent a baseline [-PL_media] concentration-response curve (CRC) to acetylcholine (ACh, 10⁻⁹ – 3x10⁻⁵ M) followed by 30-min incubation with PL_media, after which the CRC was repeated.

Results

Exposure of trophoblast cells to rotenone resulted in cell death (Vehicle: 28.17 ± 2.67% vs. Rotenone: 48.43 ± 1.22%, n = 3, P = 0.002) and mtDNA release (Vehicle: 1.69 ± 0.12 ng/uL vs. Rotenone: 2.39 ± 0.10 ng/uL, n = 5, P = 0.002). Hypoxia did not induce trophoblast cell death (Normoxia: 24.7 ± 0.50% vs. Hypoxia: 24.25 ± 0.45%, n = 2, P = 0.6), but increased release of mtDNA (Normoxia: 14.22 ± 1.20 pg/uL vs. Hypoxia: 20.64 ± 0.39 pg/uL, n = 3, P = 0.007). PL_media from normoxic and hypoxic placentae reduced sensitivity to ACh (–logEC50, Normoxia: (–)PL_media: 7.48 ± 0.03 vs. (+)PL_media: 6.96 ± 0.10, n = 4, P = 0.02; Hypoxia: (–)PL_media: 7.35 ± 0.35 vs. (+)PL_media: 6.70 ± 0.29, n = 3, P = 0.08).

Conclusions

A placental cell model of mitochondrial stress results in cell death and release of mtDNA, while a hypoxic model of stress results in release of mtDNA without cell death. Placental factors decrease resistance artery sensitivity to vasodilators in both normoxic and hypoxic conditions, indicating that the placenta contributes to maternal vascular tone in healthy pregnancies and in pregnancies complicated with reduced perfusion. Ongoing studies investigate the vasoactive potential of placenta-derived cell-free mtDNA.

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