**Effects of external factors on the trophoblast invasion: An *in vitro* comparative analysis of the transcriptome and proteome**

**Project Synopsis:**

Trophoblast invasion is a carefully controlled critical placental process under physiological hypoxia during early pregnancy. This ensures the establishment of foeto-placental unit that is needed for healthy pregnancy. Defects in this process can lead to many pregnancy related disorders such as pre-eclampsia and intrauterine growth retardation. Several studies have also implicated a link between poor trophoblast invasion and future adulthood chronic diseases (Murthi and Vaillancourt, 2018; Burton, Fowden, and Kent, 2016; Thornburg and Marshall, 2015). Interestingly several external factors such as infections (especially SARS-CoV-2), environmental pollution (such as NO2 and CO etc), maternal chronic diseases (such as diabetes, asthma etc) may also influence the trophoblast invasion. Amongst these, the adverse effects of air pollutants to human health and the developing placenta are clear, but are currently being noticed only retrospectively, after the outbreak of disease (i.e. pre-eclampsia, miscarriage/pre-term birth, low birth weight and/or developmental defects). On the other hand, the long term pregnancy related effects of SARS-CoV-2 is not fully understood. Unfortunately, due to the obvious ethical constraints, the in vivo effects of these two external factors cannot be studied in humans. Therefore the current investigations, data, and/or reports are only become apparent after the spontaneous infections and/or chronic pollutions. This highlights the importance of developing an in vitro model to study the cellular stress effects of these events

We hypothesize that air pollution, especially the particulate matters directly affects the first trimester trophoblast cell survival, proliferation and invasion. Likewise recent in silico studies and meta-analysis have suggested a possible negative long-term effects of SARS-CoV-2 infections on placental development, the invasive capacity resulting in poor placentation. (Seethy et al, 2021; Al-Lami, 2021; Allotey et al, 2020). Therefore, this proposed study sets out to characterize protein pathways underlying early trophoblast invasion using human first trimester early trophoblast cell line HTR8Svneo.

**Aims:**

The proposed study aims to determine the effect of air pollutants and the serum of individual who have recovered from SARS-CoV-2 infection on (a) the cellular toxicity/survival and elucidate the mechanisms of toxicity on the migration and invasion of trophoblast cell line HTR8Svneo; and (b) identify the effects of these events on global cellular protein/peptide expression pattern using High-throughput proteomics

**Methodology:**

The ability of the cells to survive against environmental pollutants and rejuvenated serum from after SARS-Cov-2 infection will be analysed by cell proliferation/toxicity assays including MTT, LDH and BrdU incorporation assays. Cells will be grown under normoxia (20% O2) and hypoxia (2% O2). The effects of these insults on trophoblast migration will be analysed using a scratch/wound healing assay and quantified using WimScratch-Wimesis software to show the effects of these pollutants on cell migration. For trophoblast invasion, we will use the BD-Biocoat cell invasion assay kit and calculate the number of invaded vs migrated cells using confocal microscopy and Image J software under the conditions in aim 1 to show how these pollutants may influence trophoblast invasion.

Once the toxicity profiles of these pollutants on these cells have been established, the study will focus on identifying the effects of these insults on global protein/peptide expressions. By combining the transcriptome and proteome analysis the study will aim to unveil the novel genes and their status in response to these two insults (i.e. particulate pollution and serum from individuals who have been rejuvenated after SARS-Cov-2 infections) involved in the regulation during trophoblast invasion in vitro. We plan to characterize the proteome and combine it with published transcriptome data. The proteome changes during invasion under these two “insults” will separately be characterized and compared with normal *in vitro* invasion. The data will allow the identification of potential insult-specific pathways. Given that the mRNA alone does not necessarily reflect changes at the protein level, a proteomic analysis will also be carried out using a Xevo G2-XS QTof LC/MS-MS coupled with Waters ACQUITY UPLC I-class system. Proteinlynx Global Server (PLGS) and Progenesis will be used for proteomics. Furthermore, Sequential Windowed Acquisition of all Theoretical Fragment Ion Mass Spectra (SWATH-MS) will be carried out to identify novel proteins, whose expression is affected by the exposure. This would help to identify the important functional proteins that are affected by gaseous pollution.

**Expected outcomes:**

The data from this project would assist us in providing evidence of the deleterious effects of (a) particulate pollution and (b) rejuvenated serum from after SARS-Cov-2 infections on trophoblast invasion and implantation. It would also provide important pilot data that could allow NHS health providers/carers and health care professionals to provide information to women who are pregnant or trying to conceive to prevent or minimise exposure to these insults. Above all the study would identify cellular proteins that might be affected. The novel genes/proteins identified in this study can possibly be used as biomarkers and or be useful to explore the potential drug targets on identified proteins/cell signalling pathways.

**Reference and Further Reading:**

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