Application Notes

A series of interviews conducted by HypOxygen, Don Whitley Scientific Limited's US distributor.

Dr. Houda Benlhabib is in the Department of Biochemistry at UT Southwestern in Dallas, working on the role of genetic and epigenetic mechanisms in the regulation of fetal lung development as well as in reproductive and perinatal biology.

a. *"Estrogen-Related Receptor γ (ERRγ) Regulates Oxygen-Dependent Expression of Voltage-gated Potassium (K^+) Channels and Tissue Kallikrein during Human Trophoblast Differentiation" (2013) Yanmin Luo, Premlata Kumar, and Carole R. Mendelson; Mol Endocrinol, Jun; 27(6): 940–952.*

Placental development during pregnancy is accompanied by increasing tissue oxygen levels, while persisting hypoxia contributes to the pathogenesis of pre-eclampsia and IUGR. Hypoxystation user Carole Mendelson describes studies examining the expression patterns of genes for potassium channels and tissue kallikrein under hypoxic (2% O2) conditions. She found a novel role for estrogen-related receptor ϒ (ERRϒ) as an oxygen-responsive transcription factor during trophoblast differentiation.

b. *"The c-Myc-Regulated MicroRNA-17∼92 (miR-17∼92) and miR-106a∼363 Clusters Target hCYP19A1 and hGCM1 To Inhibit Human Trophoblast Differentiation" (2013) Premlata Kumar, Yanmin Luo, Carmen Tudela, James M. Alexander, and Carole R. Mendelson; Mol Cell Biol. 2013 May; 33(9): 1782–1796.*

Mendelson's group investigated the role of miRNA's in regulating expression of genes involved in trophoblast differentiation. Oxygen tension and vascularization play a significant role in pre-eclampsia, one of the leading causes of fetal and maternal death. C-Myc expression was elevated at hypoxia, and translation of miR-17∼92 and miR-106a∼363 clusters was found to be increased, also. Together, these factors seem to promote stemness in the proliferating cytotrophoblasts and inhibit differentiation. Interesting commonalities with tumor cells with regard to proliferation and miRNA expression are discussed in this paper.

The lab has been using an Hypoxystation for about 4 years now. HypOxygen asked how the lab uses the workstation for hypoxic cell culture.

What is the focus of the research in your lab, and how is hypoxia relevant to that research?

We have been using the H35 Hypoxystation for about 4 years now. Research in Carole Mendelson's lab is focused on the developmental regulation of surfactant synthesis during fetal

lung development. We primarily study surfactant protein SP-A, the major surfactant protein produced by the type II cells which line the lung alveoli. SP-A is developmentally regulated with surfactant synthesis and, as such, is an excellent marker for type II cell differentiation. Surfactant is a phospholipid-rich lipoprotein complex; its main role is to reduce surface tension in the lung alveoli to facilitate air breathing. Humans have two SP-A genes, SP-A1 and SP-A2, and the cAMP induction of SP-A2 expression is oxygen-dependent. We are interested in examining the factors (either transcription factors or co-factors) that are regulated by oxygen tension that could potentially regulate SP-A expression. We are also interested in the effects of changes in oxygen tension on miRNA regulation and on epigenetic modifications, such as acetylation or methylation of histones which can affect SP-A gene expression either by activation or repression. We are studying histone H3K9 methylation and acetylation, as well as histone H3 lysine 4 and 27 methylation as epigenetic markers for fetal lung development. cAMP is a second messenger produced in response to extracellular signals, such as prostaglandins. Prostaglandins are ligands that promote the production of cAMP. We have observed that cAMP induction of type II cell differentiation and SP-A expression are dependent upon a critical O_2 tension in the culture environment. We are interested in defining the mechanisms by which cAMP regulates genes involved in type II cell differentiation in response to changes in oxygen tension.

Can you describe the course of a typical assay with your cells?

We obtain fetal lung tissue and process it either as an explant or we digest it using collagenase and isolate the cells on a Percoll gradient. The epithelial cells are cultured in 60 mm dishes for 24-48 hours, then harvested and lysed inside the workstation. The cells are treated with and without cAMP. We culture the lung epithelial cells at 1-2% oxygen because we are trying to mimic the oxygen tension that isphysiological for a fetus within the womb. Parallel dishes of cells are maintained at ambient oxygen tension (~20%) in an incubator, as a comparison with the hypoxia results, but we see our fetal gene expression patterns only at the very low oxygen tension. Oxygen will inhibit the cAMP induction of our gene of interest, the surfactant protein A. We extract both mRNA and protein and analyze expression patterns.

During the culture process, what are typical steps the cultures require, and how many times total would you say are you going in and out for a typical cell culture?

We change the media daily, and this doesn't disrupt the cells at all when they are in the Hypoxystation. In contrast, removing the cells from the incubator in order to change the media or to lyse the cells after culture exposes the cells to altered O_2 tension and temperature. This can create a big problem when analyzing hypoxia-induced genes. For example, HIF-1 is rapidly degraded in the presence of increased O_2 tension. With the Hypoxystation, these culturing problems don't occur, the cells stay inside the workstation and are never exposed to air.

What would you say are the advantages of the workstation as compared to a tri-gas incubator?

In the past, we used an incubator for our hypoxia work but we saw fluctuations in the gene expression patterns from one set of assays to the next, so we couldn't rely on the results. SP-A gene expression patterns were not as stable as in the workstation, which tells us the oxygen tension in the incubator just wasn't stable enough. We see a strong consistency in the results obtained with the workstation. I am very happy with the Hypoxystation, it has been very robust and we are able to work efficiently with it.

Another advantage of the Hypoxystation over an incubator is the way the humidity is controlled, it's very homogenous. Our lung epithelial cells are cultured at an air-liquid interface; thus, the cells are in actual contact with the atmosphere and can dry out if the atmospheric humidity is insufficient. We have the sterile steam humidifier in our workstation and the added humidity is what makes that air-liquid interface culture possible. It is a very stable and very clean environment.

Do you ever place animals inside the workstation or do you anticipate the need to do so?

We do work with mice, because the mouse has a surfactant gene SP-A, also. But we have never placed mice inside the workstation.

Is there any instrumentation in the w/s?

We do not have instrumentation inside the workstation currently, but we would like to have an imaging system to check on our cells.

Which conferences do you and your colleagues visit?

We always go to the FASEB lung epithelium conference, which occurs every other year. The next meeting is in 2016. We also attend the annual Endocrine Society meeting (March this year).

See more of what Houda Benlhabib is doing here:
http://www4.utsouthwestern.edu/mendelsonlab/The_Mendelson_Lab/Welcome.html