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Elucidating the effect of known CYP3A inducers Vitamin D3 and Valproic Acid on the differentiation of iPSC-derived enterocyte-like cells

Introduction

Despite being highly regenerative in vivo, primary human intestine epithelial cells undergo rapid apoptosis in vitro and cannot persist in long-term culture, resulting in the need for a suitable substitute for drug development and disease modelling. Highly proliferative human induced pluripotent stem cells (iPSCs) have been successfully differentiated into all major intestine epithelial cell types and provide an alternative to primary cells. In Ogaki et al., 2013, Kume-Shiraki laboratory reported the differentiation of human embryonic stem cells (hESCs) into the four major intestine epithelial cell types induced by BIO and DAPT treatment on the M15 feeder layer, confirming that Wnt and Notch signals governed the differentiation of hESC into intestinal lineages. This protocol was then adapted into a directed differentiation system for enterocytes with the addition of maturation medium M3 with BIO, Dexamethasone, DAPT and activated Vitamin D3 (VD3) to improve drug transporter and metabolic enzyme gene expression and functionality on the novel Collagen Vitrigel Membrane (CVM) in a culture system specialized for pharmacokinetic studies. However, aside from drug transporter and xenobiotic-focused metabolic enzyme functionality, other intestine-related functions were not looked at, nor were the specific changes of inducer VD3 on our cells investigated.

In this dissertation, bulk RNA-sequencing methods were employed to investigate the changes in gene expression induced by metabolic enzyme Cytochrome P40 (CYP) inducer VD3, as well as the effects of Notch activator and known CYP modulator Valproic Acid (VPA) in promoting function-related gene expression and maturity of our iPSC-derived enterocytes.

Results and Discussion

1. Notch, Wnt and BMP signaling regulate the differentiation of iPSCs into absorptive enterocyte-like cells

To analyse if the differentiation process of iPSC-derived enterocytes in our established protocol echoes that of in vivo processes, qPCR and bulk RNA sequencing analysis was carried out on samples at undifferentiated (D0), precursor (D10, D15) and mature (D23) samples. We confirmed that the in vitro differentiation of iPSCs to iPSC-derived enterocyte-like cells on CVM demonstrated the expected functions as reported in developmental studies, suggesting that iPSCs provide a suitable platform to study the differentiation process of enterocytes in vivo. In summary, genes regulating general cellular processes are downregulated upon differentiation, whilst intestine-specific gene expressions are upregulated upon differentiation, and heatmaps illustrate the obvious changes in Notch, BMP and Wnt pathways throughout the differentiation timeline. Whilst BMP signaling promotes enterocyte maturation, Notch signaling could also promote enterocyte differentiation by inhibiting transcription factors for secretory fates.

2. VD3 induces CYP3A4 and functional specification in iPSC-derived enterocyte-like cells

The inclusion of VD3 in M3 maturation media not only induces the expression and activity of CYP3A4, but also has the effect of promoting enterocyte functional specification to that characteristic of villus-tip enterocytes, likely due to BMP-signaling mediated by VD3. BMP signaling directly regulates the functionality of mouse enterocytes, upregulating fatty acid metabolism-related genes apolipoproteins and downregulating and sugar metabolizing genes (Buemer et al., 2022). These reports were in-line with our results, where VD3 enriches Gene Ontology (GO) categories related to fatty acid metabolism, such as bile transporter activity, digestive processes, and lipid translocation. We also noted a significant upregulation of *BMP2* transcript levels, a potent modulator of the apolipoprotein-coding genes, as well as modulator *SMAD4* and BMP receptor *BMPR2* with VD3 treatment.

3. VPA activates Notch signaling and upregulates expression of intestine function-related genes

We next attempted to elucidate the effect of VPA, an HDAC inhibitor, Notch activator and known CYP3A4 regulator on the maturation of iPSC-derived enterocytes in M3 medium. VPA was found not to exert much significant effect in the absence of VD3 but worked synergistically with VD3 to significantly improve gene expression of intestine functional genes such as those in GO categories aquaporins, fatty-acid degradation and vitamin digestion and absorption. VPA treatment in tandem with VD3 also upregulated key regulators of enterohepatic circulation such as *FGF19* and basolateral bile acid transporters *SLC51A* and *SLC51B*. Interestingly, VPA treatment instead downregulated expression of apical bile acid uptake transporter *SLC10A2*, which could be attributed to increased sensitivity of iPSC-derived enterocytes to bile acid levels in culture medium as a result of enriched levels of regulator *FGF19*. To sum up, we were able to ascertain the genetic profile of our D23 iPSC-derived enterocyte-like cells to be specialized towards fatty acid absorption and metabolism instead of absorption and metabolism of carbohydrates and other nutrients. These results suggest a possibility for our VD3VPA-treated iPSC-derived enterocyte-like cells for use in a co-culture model with hepatocytes to model enterohepatic circulation *in vivo*.