Title: Physioxia-driven maturation of iPSC-derived hepatic cells

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Abstract

In the human liver, hepatocytes, the main cell type of the liver, are arranged in units called lobules, each further divided into three metabolically distinct zones. This zonation can be attributed to the presence of various gradients, such as glucose and oxygen gradients. Notably, drug metabolism, one of the liver's most crucial functions, is mediated by cytochrome P450 enzymes, which are predominantly expressed in the least oxygenated zone of the lobules.

Our study highlights the often-overlooked role of physiological oxygen levels in the differentiation and culture of induced pluripotent stem cell (iPSC) derived cells. While efforts are made to optimise aspects of cell culture environments, such as media composition, oxygen levels are frequently neglected, despite the stark contrast between atmospheric oxygen levels (~21%) and tissue-specific physioxia. In the liver, oxygen concentrations range from approximately 4% to 10% along the lobule axis.

To better replicate the liver's natural microenvironment, we differentiated iPSC-derived hepatocytes under physiologically relevant oxygen conditions. Our analyses indicate increased secretion of liver-specific proteins along with up-regulated expression of key hepatic genes, including members of the P450 family, even in the absence of media perfusion or 3D culture conditions.

Our findings demonstrate that adjusting a single physical parameter, such as oxygen levels, can significantly enhance the functionality of iPSC-derived hepatocytes. This simple yet impactful approach underscores the importance of mimicking physiological oxygen levels to support the liver's complex functions and offers a promising strategy for advancing liver research and developing more advanced and physiologically accurate *in vitro* liver models.