Modelling the Liver Microenvironment: Development and Characterisation of Ruminant Liver Organoids to Investigate Host-Parasite Interactions

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Understanding how helminth parasites modulate host responses within the liver has been limited by the lack of physiologically relevant models, particularly for tissue-resident liver cell populations. Organoid-based technologies offer a powerful approach to interrogate species- and tissue-specific host–parasite interactions. Liver organoids have previously been derived from other species, however, to date these models have not been adapted for ruminants, specifically cattle and sheep. Here, we describe the development and characterisation of ruminant three-dimensional (3D) liver organoids for modelling cellular and molecular interactions between the liver fluke *Fasciola hepatica* and the host liver. Intrahepatic ductal tissue was digested to release progenitor-cell containing hepatic ductal fragments. When initially established and cultured in organoid growth media (OGM), organoids from both species were comprised of KRT19- and KRT18-positive cholangiocytes. We then evaluated the capacity for organoids to differentiate into hepatocyte-enriched cultures and noted increased hepatocyte markers in bovine cultures.

A comprehensive analysis of the liver tissue and organoids between the species revealed species-specific differences in gene expression, which were conserved within organoid cultures. Alongside this, we demonstrate here that organoids from both species were capable of metabolising drugs into active metabolites, using the anthelminthic Triclabendazole as a test compound. We then aim to challenge the developed ruminant liver organoids with the excretory/secretory products from *F. hepatica* to investigate the complex host-parasite interactions occurring at the parenchymal liver cells using transcriptomic analysis. Overall, this study provides insights into differences in liver composition and function between ruminant species, as well as providing novel experimental models for identifying parasite-derived drivers of pathology and informing vaccine target discovery.