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Characterization of stem cell-derived liver and intestinal organoids as a model system to study nuclear receptor biology

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ABSTRACT

Nuclear receptors (NRs) are ligand-activated transcription factors regulating a large variety of processes involved in reproduction, development, and metabolism. NRs are ideal drug targets because they are activated by lipophilic ligands that easily pass cell membranes. Immortalized cell lines recapitulate NR biology poorly and generating primary cultures is laborious and requires a constant need for donor material. There is a clear need for development of novel preclinical model systems that better resemble human physiology. Uncertainty due to technical limitations early in drug development is often the cause of preclinical drugs not reaching the clinic. Here, we studied whether organoids, mini-organs derived from the respective mouse tissue's stem cells, can serve as a novel model system to study NR biology and targetability. We characterized mRNA expression profiles of the NR superfamily in mouse liver, ileum, and colon organoids. Tissue-specific expression patterns were largely maintained in the organoids, indicating their suitability for NR research. Metabolic NRs *Fxrα*, *Lxrα*, *Lxrβ*, *Pparα*, and *Pparγ* induced expression of and binding to endogenous target genes. Transcriptome analyses of wildtype colon organoids stimulated with Rosiglitazone showed that lipid metabolism was the highest significant changed function, greatly mimicking the PPARs and Rosiglitazone function *in vivo*. Finally, using organoids we identify *Trpm6*, *Slc26a3*, *Ang1*, and *Rnase4*, as novel Fxr target genes. Our results demonstrate that organoids represent a framework to study NR biology that can be further expanded to human organoids to improve preclinical testing of novel drugs that target this pharmacologically important class of ligand activated transcription factors.
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1. Introduction

Nuclear receptors (NRs) represent a superfamily of ligand-dependent transcription factors which regulate a large variety of processes, including reproduction, development, metabolism, and immune responses [1]. NRs are thought to be an important class of drug targets, as their ligands are lipophilic and easily pass biological membranes. In addition, NRs regulate entire pathways. It has been estimated that 13% of all FDA-approved drugs target NRs [2]. Further fine-tuning of NR ligand specificities is needed to diminish cross-reactivity, and side-effects of existing NR agonists. More potent, selective, and tissue specific agonists/antagonists need to be identified and developed for their optimal use in the clinic. Rational design of such ligands warrants better insights in the exact molecular mechanisms of NR action, since these are largely unknown. Investigations in tumor-derived cell lines are hampered by poor expression of NRs and their target genes [3], and most studies on NR molecular mechanisms are based on overexpression studies. These tumor cell lines were selected to survive harsh environments, and are frequently characterized by high expression of anti-apoptotic proteins, such as Bcl-2 family members [4]. As a consequence, this may result in increased resistance to pharmacological drugs, and therefore not representative of the sensitivity of tissue. Primary cultures are a good alternative, however, these require a continuous supply of donor material. Sato and Clevers first described the isolation and maintenance of intestinal organoids, so-called mini-guts a few years ago [5]. In the meantime, organoids from several mouse and human tissues, *e.g.* liver, colon, pancreas, prostate, and stomach have been generated [6–9], and have been shown to be a powerful study tool. Organoids are 3D epithelial primary cell cultures derived from Lgr5^{+} stem cells which strongly recapitulate organ physiology and structure. The main

Abbreviations: ChIP, chromatin immunoprecipitation; DIC, differential interference contrast; DM, differentiation medium; DSG, Di(*N*-succinimidyl) glutarate; EM, expansion medium; Fxr, farnesoid x receptor; IPA, Ingenuity Pathway Analysis; Lxr, Liver x receptor; NR, nuclear receptor; OCA, obeticholic acid; Ppar, Peroxisome proliferator-activated receptor; RMA, robust multiarray analysis; Rosi, Rosiglitazone.

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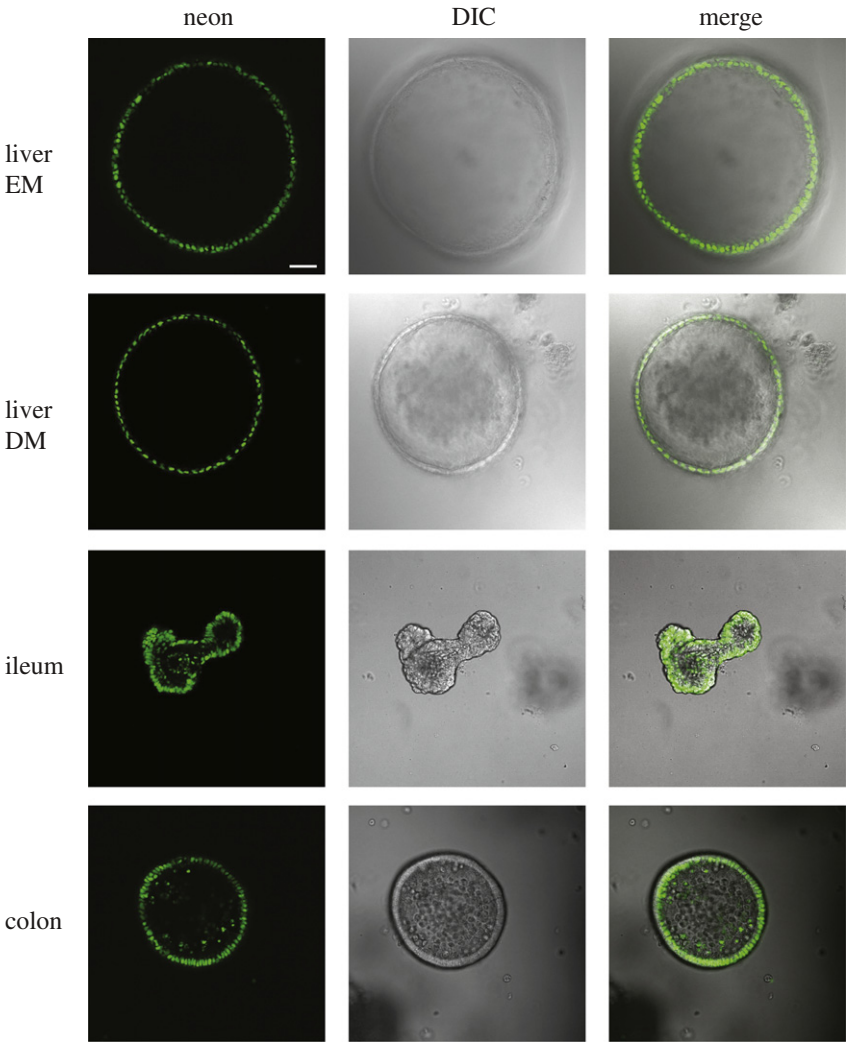

Fig. 1. Morphological representation of liver, ileum, and colon organoids. Liver, ileum, and colon organoids derived from WT mice were transduced with an hH2B-neon construct to overexpress histone H2B. Liver organoids were cultured in expansion (EM) or differentiation medium (DM). Magnification 20×; scalebar: 50 μm; Neon: hH2B-neon expression, green staining in nucleus; DIC: differential interference contrast.

advantage is the requirement for only one small piece of primary tissue, such as a biopsy, for setting up an organoid culture, which can be expanded while maintaining intact stem cell compartments during long-term culture, in addition the cultures are genetically stable over time, and organoids can be easily manipulated using techniques similar to those used in cell line cultures. Notably, Huch et al. recently showed that the human liver organoids showed stronger hepatocyte functions when compared to the reference cell line HepG2, including bile acid production, and Cyp3A4 activity [10], which are regulated by NRs. Microarray analysis of liver organoids revealed that organoids resemble their corresponding adult tissue [11]. By changing medium components, *i.e.* inhibition of Notch and TGFβ signaling by withdrawal of R-spondin, HGF, and nicotinamide, and addition of A8301 and DAPT, hepatocyte maturation can be induced *in vitro*. Genes involved in cholesterol and lipid metabolism are induced in differentiated liver organoids [11], indicating that NRs must be expressed in these organoids upon differentiation. Also for intestinal organoids, it has been shown that organoids are highly similar to the adult intestinal epithelium, with stem cells that differentiate into all known epithelial cell lineages in a polarized manner [5,6].

The entire NR transcriptome has been studied in mouse tissues, revealing a complex hierarchical transcriptional regulatory network [12]. This raised the question whether this network is preserved in organoids. Therefore, we characterized the mRNA expression profile of the NR superfamily in mouse liver, ileum, and colon organoids. We show that organoids express most NR family members, and these largely resemble the mRNA expression pattern in the respective tissues. For the selected metabolic NRs *Fxrα*, *Lxrα*, *Lxrβ*, *Pparα*, and *Pparγ*, target genes were induced upon stimulation by specific agonists, showing that organoids are responsive to NR activation. Genome-wide analysis

Fig. 2. NRs are differentially expressed in organoids. Basal mRNA expression of 50 NRs was determined by qRT-PCR in mouse liver undifferentiated (EM; A) and differentiated (DM; B), ileum (C), and colon organoids (D). Relative mRNA expression values are shown. One representative experiment in technical duplicate of one male mouse per organoid line is shown. Each bar represents mean ± SD. Expression is ranked from high to low. Black bars represent NRs which are expressed at a cycle time (Ct)-value of <33; grey bars Ct-value 33–40. Ct-value >40: mRNA expression is undetectable. Dotted lines indicate the separation between the two y-axis scalebars. Venn diagram (in B) shows that 3 NRs are exclusively expressed (*i.e.* Ct <33) in EM, 7 only in DM, and 20 NRs are expressed in both EM and DM liver organoids. (E) Heatmap showing organoid NR expression patterns. LE: liver EM organoids; LD: liver DM organoids; IO: ileum organoids; CO: colon organoids.

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