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Research Article

Hepatic differentiation of human pluripotent stem cells on human liver progenitor HepaRG-derived acellular matrix

Liisa K. Kanninen [a], Pauliina Porola [a], Johanna Niklander [a], Melina M. Malinen [a], Anne Corlu [b], Christiane Guguen-Guillouzo [b], Arto Urtti [a,c], Marjo L. Yliperttula [a], Yan-Ru Lou [a,*]

[a] Centre for Drug Research, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland
[b] Inserm UMR991, Liver Metabolisms and Cancer, Université de Rennes 1, F-35043 Rennes, France
[c] School of Pharmacy, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

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ABSTRACT

Human hepatocytes are extensively needed in drug discovery and development. Stem cell-derived hepatocytes are expected to be an improved and continuous model of human liver to study drug candidates. Generation of endoderm-derived hepatocytes from human pluripotent stem cells (hPSCs), including human embryonic stem cells and induced pluripotent stem cells, is a complex, challenging process requiring specific signals from soluble factors and insoluble matrices at each developmental stage. In this study, we used human liver progenitor HepaRG-derived acellular matrix (ACM) as a hepatic progenitor-specific matrix to induce hepatic commitment of hPSC-derived definitive endoderm (DE) cells. The DE cells showed much better attachment to the HepaRG ACM than other matrices tested and then differentiated towards hepatic cells, which expressed hepatocyte-specific makers. We demonstrate that Matrigel overlay induced hepatocyte phenotype and inhibited biliary epithelial differentiation in two hPSC lines studied. In conclusion, our study demonstrates that the HepaRG ACM, a hepatic progenitor-specific matrix, plays an important role in the hepatic differentiation of hPSCs.

1. Introduction

Human embryonic stem cells (hESCs) [1] and human induced pluripotent stem cells (hiPSCs) [2,3] have the ability to form all cell types of an adult body; thus they are highly interesting sources of cells for various human cell-based applications in the field of drug research. Hepatocytes are needed in drug discovery and development for prediction of biotransformation pathways, possible drug–drug interactions, and hepatotoxicity of a drug candidate. Differentiation of both hESCs and hiPSCs to hepatocyte-like cells has been studied broadly [4–8]; but still the biggest challenge is to obtain mature hepatocyte-like cells [9,10]. In the majority of the currently used differentiation protocols, stem cell fate is guided towards hepatocyte-like cells solely by a stepwise growth factor treatment. It is, however, known that not only soluble factors but also cell–cell interactions and cell–matrix interactions play an important role in the complex, multistage hepatic cell differentiation process [11].

Biomaterials mimicking the extracellular matrix (ECM) are ideal for *in vitro* cell culturing since they provide closely tissue-resembling microenvironments for cultured cells. ECM-mimicking *in vitro* culture systems can be established by using natural biomaterials, such as decellularized matrix, also called acellular matrix (ACM) [12] and ECM components, and synthetic biomaterials, such as synthetic polymeric hydrogels. Decellularized whole organs are the most natural, simulating scaffolds since tissue microarchitecture and its components such as proteins, glycosaminoglycans, and growth factors can be well maintained [13]. These scaffolds are useful in whole organ engineering [14], but due to demanding decellularization and repopulation processes using perfusion systems, they are not optimal matrices for *in vitro* high-throughput drug testing during drug development. In addition, whole organ ACM scaffolds cannot mimic a defined region of a tissue to induce cell type-specific effects.

During embryogenesis, the cells from definitive endoderm (DE) differentiate to a fully functional adult hepatocyte fate under specific matrices and hormonal conditions [15]. To mimic the *in vivo* differentiation process, *in vitro* hepatic differentiation most commonly starts from DE cells. The soluble factors used during *in vitro* hepatic differentiation have been given most of the focus of research efforts because they are available commercially in

* Corresponding author.
E-mail address: yan-ru.lou@helsinki.fi (Y.-R. Lou).

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purified forms and can be easily added into culture media. Contrary, the optimal matrix for *in vitro* hepatic commitment of the DE cells is poorly understood, partially due to the complexity of matrix and unavailability of specific matrix components. Here we show that human liver progenitor cell-derived ACM supports the attachment of DE cells and their hepatic differentiation.

2. Materials and methods

2.1. Preparation of the ACM

We prepared the ACM from human liver progenitor HepaRG cells. The HepaRG cells [16] were obtained from Biopredic (Saint-Grégoire, France), and they were plated at 26,000 cells/cm^2 density and cultured for two weeks in previously prescribed culture conditions during which the cells are known to differentiate to hepatocyte-like cells and cholangiocyte-like cells through a bipotent progenitor [17,18]. Decellularization was performed as

A

Supplement to RPMI-1640 medium	M1	M2	M3	M4
1 x GlutaMAX (Gibco, 35050-061)	x	x	x	x
1 x B-27 (Gibco, 17504-044)	x	x	x	x
100 ng/ml Activin A (PeproTech, 120-14E)	x	x		
100 ng/ml Activin A (Gibco, PHG9014)			x	x
75 ng/ml Wnt-3a (R&D Systems, 5036-WN-010/CF)	x		x	
1 mM (day 0) and 0.5 mM (days 1-5) Sodium butyrate (Sigma-Aldrich, B5887)	x		x	

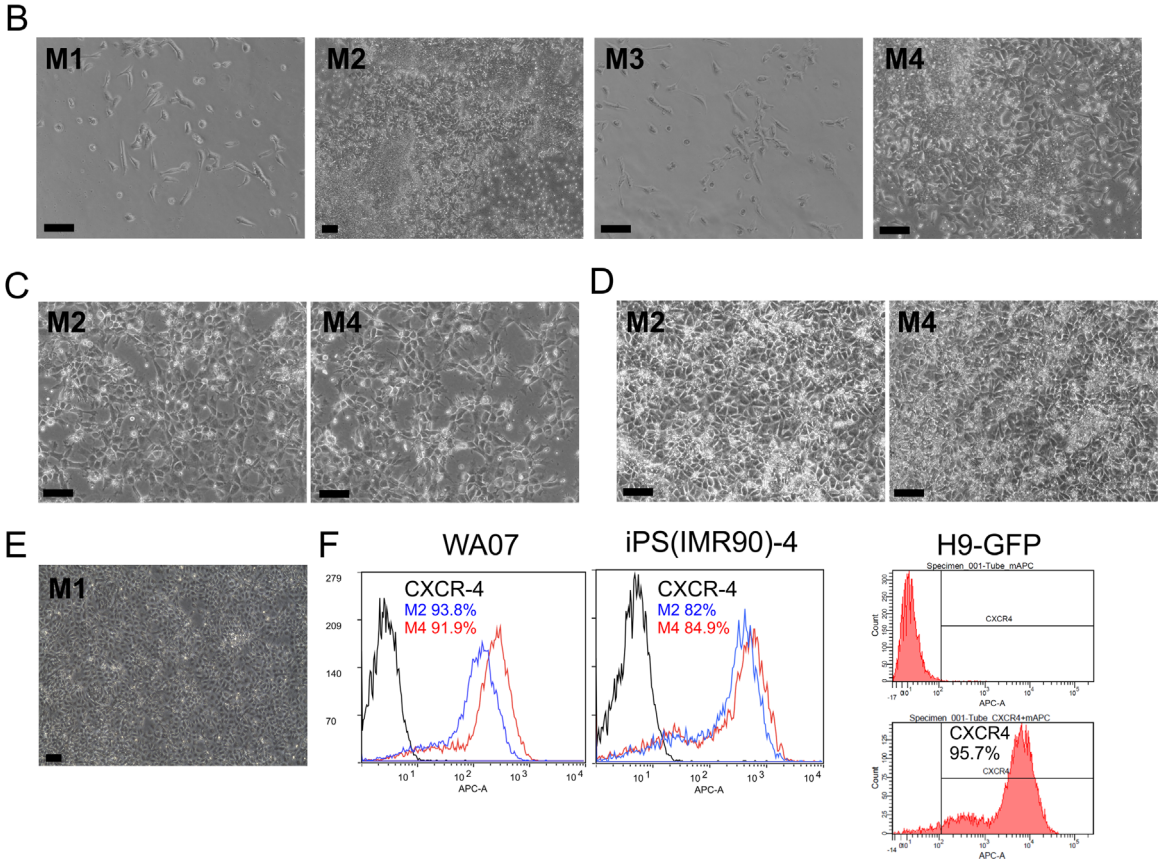

Fig. 1. Optimization of the medium components for the DE induction. (A) The reagents used to supplement RPMI-1640 medium for the DE induction media M1, M2, M3, and M4. (B) The iPS(IMR90)-4 cells in the DE induction media on day 2. (C) The iPS(IMR90)-4 cells after 6 days in the M2 and M4 media. (D) The WA07 cells after 6 days in the M2 and M4 media. (E) The H9-GFP cells after 6 days in the M1 medium. Scale bars = 100 μm. (F) Flow cytometry analysis of the *CXCR-4* expression in the WA07 and iPS(IMR90)-4-derived cells after 6 days in the media M2 and M4 and H9-GFP-derived cells after 6 days in the medium M1.