Precursor and interstitial Cajal cells in the human embryo liver

MUGUREL CONSTANTIN RUSU1, IRINA DUTA2, ANDREEA CRISTIANA DIDILESCU3, ALEXANDRA DIANA VRAPCIU1, SORIN HOSTIUC4, EMIL ANTON5

1) Discipline of Anatomy, Faculty of Dental Medicine, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
2) Discipline of Physiopathology II, Department 2, Faculty of Medicine, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
3) Discipline of Embryology, Faculty of Dental Medicine, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
4) Discipline of Legal Medicine, Faculty of Medicine, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
5) Department “Mother and Child”, Faculty of Medicine, “Grigore T. Popa” University of Medicine and Pharmacy, Iassy, Romania

Abstract

Interstitial Cajal Cells (ICCs) were only proven in human adult hepatic tissue. The immune phenotypes of various cell types in the human embryonic liver (HEL) are scarcely described. It was hypothesized that in HEL ICCs are present and distinctive to the precursor/progenitor cells populations. It was aimed and performed a qualitative study of HEL by use of antibodies against CD117/c-kit, CD31, CD34, CD90, CD105, DOG1, Ki67, and adiponectin. Five human embryos of 23–29 mm were used. Blasts and hematopoietic cells were comprising the two major cell populations in late stage embryo. The general population of blasts in the HEL was CD34+/CD105, although scarce CD117/c-kit+ and CD90+ such cells were found. Hematopoietic precursors were Ki67+. Adiponectin-positive plasmalemmas were found mostly in blasts. Endothelia were CD31+/CD34+. Interstitial cells with moniliform prolongations were found; such cells were scarcely CD117/c-kit+ but consistently DOG1+. They were diagnosed as ICCs but based on the morphology of their prolongations they can be equally viewed as being telocytes (TCs). Further studies should better correlate the precursor cell-types and immune phenotypes during human liver organogenesis. Liver ICCs and/or TCs should be also investigated in the human fetal liver.

Keywords: adiponectin, CD117, c-kit, anoctamin 1, DOG1, Ki67, CD105, CD90, telocytes, telopodes.

Introduction

Interstitial Cajal cells (ICCs) are mesenchyme-derived cells, which are distributed throughout the gastrointestinal tract, as well as in other smooth muscle tissues [1, 2]. These cells are either involved in inhibitory neuro-transmission or play a pacemaker role [3–5], or are part of the intestinal stretch receptor [6]. ICCs express the receptor tyrosine kinase c-kit [5, 7]. However, c-kit is not a specific marker for ICCs, as it also labels stem/progenitor cells, neurons, glia, melanocytes and mast cells [7–17]. The c-kit positivity may however not be an essential criterion for the ICCs [8]. Anoctamin 1 (Ano1, commonly known as DOG1) is highly specific for all classes of ICCs [1], and was found labeling esophageal ICCs in human embryos [18]. Ano1 was detected in multipolar ICCs of the myenteric plexus and in bipolar cells of the circular muscle layer [19].

In humans there were identified intrahepatic ICCs in the portal spaces and septa [20]. It is actually considered that during development, liver stem cells (LSCs) differentiate into hepatocytes and cholangiocytes, but little is known about the molecular dynamics in LSCs [21]. Adult LSCs were found c-kit-negative and CD45/TER119 negative [22]; and studies performed in vitro concluded that c-kit inhibitors do not affect the proliferation of cultured LSCs [23]. However, little is known regarding the nature of the hepatic progenitor cells and whether an equivalent to the adult LSCs exists in the fetal liver [24, 25].

The anlage of the liver arises from the endoderm of the foregut (supplying the LSCs), the mesenchyme of the septum transversum, the lining of the major blood vessels in the septum transversum (it supplies the lining cells of the hepatic sinusoids), the blood islands of the yolk sac (partly supplying the hematopoietic tissue) [26]. At the beginning of the liver organogenesis, changes occur in the metabolism of the differentiating cells: the hepatocytes, and five basic types of mesenchyme-derived cells: hematopoietic, endothelial, fibroblasts, smooth muscle cells (SMCs) and macrophages [27].

It was therefore hypothesized that in human late stage embryo, also intrahepatic ICCs are present, distinctive to precursor/progenitor cells and it was thus aimed at performing a qualitative immunohistochemical study by use of CD117/c-kit and DOG1 antibodies. Additional antibodies were designed to evaluate the precursor cells of the embryonic liver and thus to support the ICCs diagnosis.

Materials and Methods

Five human embryos, which resulted from legal abortions, were collected immediately postabortion. The lengths of these embryos varied between 23 and 29 mm, thus corresponding to a 54–56 days [28] embryonic stage.

ISSN (print) 1220–0522      ISSN (on-line) 2066–8279
Approval for the present study was granted by the Ethics Committee of the “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania.

Samples were fixed for 24 hours in buffered formalin (8%) and were processed with an automatic histoprocessor (Diapath, Martinengo, BG, Italy) with paraffin embedding. Sections were cut manually at 3 μm, and were mounted on SuperFrost electrostatic slides for immunohistochemistry (Thermo Scientific, Menzel–Gläser, Braunschweig, Germany).

Histological evaluations used 3 μm thick sections stained with Hematoxylin and Eosin.

Primary antibodies for CD117/c-kit (clone T595, Novocastra-Leica, Leica Biosystems Newcastle Ltd., Newcastle Upon Tyne, UK, 1:20), DOG1 (clone K9, Novocastra-Leica, Leica Biosystems Newcastle Ltd., Newcastle Upon Tyne, UK, 1:100), CD34 (clone QBEnd10, Dako, Glostrup, Denmark, 1:50), CD31 (clone JC70A, Dako, Glostrup, Denmark, 1:50), Ki67 (clone MIB-I, Dako, Glostrup, Denmark, 1:50), adiponectin (clone 19F1, Abcam, Cambridge, UK, 1:20), CD105 (polyclonal, Thermo Scientific, Pierce Biotechnology, Rockford, USA, 1:50), CD90/Thy1 (clone EPR3132, Novus Biologicals Ltd., Cambridge, UK, 1:100) were used.

Sections were deparaffinized, rehydrated and rinsed in TBS buffer solution at pH 7.6 (for the CD117/c-kit and DOG1 antibodies) and in PBS buffer solution at pH 7.4 (for the other antibodies). Retrieval by incubation in specific buffer was completed as follows: (a) for CD34: EDTA, pH 9; (b) for the other antibodies: 0.01 M citrate retrieval solution, pH 6. The standard ABC technique used a DAB protocol. Appropriate blocking of endogenous peroxidase was completed before immune labeling (Peroxidase 1, Biocare Medical, Concord, CA, USA).

Sections incubated with non-immune serum served as negative controls. Sections were counterstained with Hematoxylin.

The microscopic slides were analyzed and micrographs were captured and processed using a Zeiss working station, as previously described [29].

Results

In late stage human embryo liver samples, the general cell population was composed of hematopoietic precursors and liver precursor/stem cells (blasts) (Figure 1), and the lobular structure of the liver had not yet established. Endothelia of hepatic microvessels were positively labeled with anti-CD31, anti-CD34, anti-CD105 and anti-adiponectin antibodies. There were no non-endothelial cells labeled with CD34 antibodies (Figure 1B).

Hematopoietic precursors were Ki67-positive (Figure 1C). Embryonic LSCs, but not hematopoietic precursors, were positively labeled (plasmalemmal labeling) by anti-adiponectin antibodies (Figure 1D). Embryonic LSCs were CD105-positive (Figure 1E). However, CD117/c-kit positive progenitor cells were scarcely found (Figure 1F), as also were CD90-positive progenitors (Figure 2).

Antibodies against CD117/c-kit also labeled cells with moniliform prolongations scarcely intermingled with progenitor cells (Figure 3). Intermingled with the general population of progenitor cells there were also found DOG1-positive multipolar cells (Figure 4). No other cells, hematopoietic or precursor, lacking processes, were DOG1-positive.

Discussion

The present study led to two different major findings, previously unreported in human embryos. The phenotype of embryonic progenitor/stem cells was mostly CD34-/CD105-, although scarce CD117/c-kit+ and CD90+ cells were also found. On other hand, interstitial Cajal cells were assessed in the embryonic liver, based on their morphology and the CD117/c-kit+/DOG1+ immune phenotype.

The available data on the embryonic liver precursor cells phenotypes are scarce, although few studies were focused on fetal, but not embryonic hepatic tissue. Progenitor c-kit expressing cells were isolated from human fetal liver, by use of immunoadherence to a monoclonal antibody (SR-1) against human kit receptor [30]. Cultured adherent SR-1 cells coexpressed progenitor markers, such as CD34, while the non-adherent SR-1 cells were morphologically recognizable precursor cells in which the kit receptor was not detectable [30]. Isolated CD117+/CD34+/CD90- cells of the fetal liver also expressed genes and proteins for hepatic markers [24]. Different to in vitro studies, we found here only scarce CD117/c-kit+ and CD90+ progenitors, most of the precursor population of the embryonic liver being CD117/c-kit-/CD34-/CD90-. This in vivo embryonic phenotype indicates that a switch could occur further in fetal liver. Although we scarcely found CD117/c-kit+ blasts, doubts should be kept whether or not these cells belong to a hematopoietic precursor population, or to embryonic LSCs, being known that c-kit rather labels hematopoietic progenitors [31].

Noteworthy, in mice embryos was described a population of c-kitlow/CD45- progenitor cells involved in liver organogenesis [32]. C-kit and its ligand, the Stem Cell Factor (SCF), are not indispensable for the proliferation of hepatic progenitors thus a direct correlation between c-kit- and Ki67 labeling was not established, neither in vitro [23], nor in this in vivo study.

Adipokines play a role in fetal development and metabolism, although it is not yet clear if they can stimulate fetal tissues development acting as growth factors [33]. Adiponectin plays a role in preimplantation embryo development in a paracrine/autocrine manner [34], has a paracrine/autocrine effect in liver regeneration and acts as a stem cell factor by regulating proliferation of stem cells [35–38]. In this regard, the adiponectin-positive phenotype of the blasts in the embryonic liver could be viewed as a normal feature, as much as adiponectin may enhance the proliferation of hematopoietic stem cells (HSCs) [38]. Moreover, LSCs in children and adolescent liver were also found positive for adiponectin [39] while adiponectin deficiency impairs liver regeneration [40].
Precursor and interstitial Cajal cells in the human embryo liver

Figure 1 – Embryonic liver in a 27 mm human embryo. Immune labeling for CD31 (A), CD34 (B), Ki67 (C), adiponectin (D), CD105 (E) and CD117/c-kit (F). There are indicated: hematopoietic foci (white arrows), microvessels (white arrowheads), embryonic LSCs (black arrows). In (F), a CD117/c-kit positive progenitor/stem cell (black arrowhead) is identified.

Figure 2 – Embryonic liver in a 27 mm human embryo. CD90-positive progenitor cells are scarce (inset, arrow).
c-kit positive ICCs was assessed [42]. The expression of the c-kit ligand gene in liver diminishes after the 15th embryonic day and its expression in embryonic liver is related to hematopoiesis [43]. These could explain why only scarce CD117/c-kit positive ICCs were found here in the late stage embryonic liver. However, the DOG1+ phenotype of these strongly supports the ICC cell-type diagnosis. The discussion goes beyond identification of ICCs in the embryonic liver because a new type of stromal cells was recently described, the telocyte (TC) [44]. Telocytes were previously considered as being interstitial Cajal-like cells (ICLCs) [45–48]. Actually, TCs are defined on a morphological basis, as “cells with telopodes” [49, 50], which, in turn, are long, slender and moniliform prolongations consisting of dilations named podoms and thin segments – the podomers [51]. A CD117/c-kit+ and/or CD34+ phenotype of TCs is usual, although non-specific [14, 49, 52–58]. In this regard, the ICCs we demonstrated in embryonic liver could be regarded as DOG1+/CD117low/CD34+ TCs. This is reinforced by few studies, which found that interstitial cells in embryo are initially CD34+, they further acquire a CD34+ phenotype, as well as fibroblastic features, then they reach after birth a TC ultrastructure but keep a limited CD34 positivity [49]. Noteworthy, hybrid morphologies mimicking TCs can only be identified in transmission electron microscopy [59].

Conclusions

Further studies should better correlate the precursor cell-types and immune phenotypes during human liver organogenesis. Liver ICCs and/or TCs should be investigated also in the human fetal liver.

Acknowledgments

This study was supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/107/1.5/82839 (author #2).

All authors have contributed equally to this study.

References


Corresponding author
Mugurel Constantin Rusu, Associate Professor, MD, PhD, Dr. Hab., Discipline of Anatomy, Faculty of Dental Medicine, “Carol Davila” University of Medicine and Pharmacy, 8 Eroilor Sanitari Avenue, 050474 Bucharest, Romania; Phone +40722–363 705, e-mail: anatomon@gmail.com

Received: November 25, 2013

Accepted: March 29, 2014