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# Identification and Characterization of Mesenchymal-Epithelial Progenitor-Like Cells in Normal and Injured Rat Liver

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In normal rat liver, thymocyte antigen 1 (Thy1) is expressed in fibroblasts/myofibroblasts and in some blood progenitor cells. Thy1-expressing cells also accumulate in the liver during impaired liver regeneration. The origin and nature of these cells are not well understood. By using RT-PCR analysis and immunofluorescence microscopy, we describe the presence of rare Thy1$^+$ cells in the liver lobule of normal animals, occasionally forming small collections of up to 20 cells. These cells constitute a small portion (1.7% to 1.8%) of nonparenchymal cells and reveal a mixed mesenchymal-epithelial phenotype, expressing E-cadherin, cytokeratin 18, and desmin. The most potent mitogens for mesenchymal-epithelial Thy1$^+$ cells *in vitro* are the inflammatory cytokines interferon γ, IL-1, and platelet-derived growth factor-BB, which are not produced by Thy1$^+$ cells. Thy1$^+$ cells express all typical mesenchymal stem cell and hepatic progenitor cell markers and produce growth factor and cytokine mRNA (Hgf, Il6, Tgfa, and Tweak) for proteins that maintain oval cell growth and differentiation. Under appropriate conditions, mesenchymal-epithelial cells differentiate *in vitro* into hepatocyte-like cells. In this study, we show that the adult rat liver harbors a small pool of endogenous mesenchymal-epithelial cells not recognized previously. In the quiescent state, these cells express both mesenchymal and epithelial cell markers. They behave like hepatic stem cells/progenitors with dual phenotype, exhibiting high plasticity and long-lasting proliferative activity. *(Am J Pathol 2015, 185: 110—128; http://dx.doi.org/10.1016/j.ajpath.2014.08.029)*

The liver has a remarkable capacity to regenerate. In rats, after partial hepatectomy (PH), the resident cells (hepatocytes, biliary epithelial cells, Kupffer cells, stellate cells, and endothelial and sinusoidal cells) undergo one or two rounds of cell division and restore the liver mass in 7 to 10 days.[1] However, when hepatocyte function is compromised and coupled with inability of residual hepatocytes to proliferate, the liver restores its mass through oval cell (OC)—mediated liver regeneration. OCs behave like adult hepatic progenitor cells; they proliferate and differentiate into hepatocytes and cholangiocytes.[2–10] OCs form pseudoducts that are in close proximity to desmin-positive cells.[11] Because OCs and thymocyte antigen 1 (Thy1)/desmin-expressing cells are in close contact, Thy1 was proposed as a marker of hepatic OCs.[12] However, subsequent publications reported that in rat liver, after 2-acetylaminofluorene treatment in conjunction with PH (2-AAF/PH), Thy1 is expressed in hepatic myofibroblasts.[13,14]

Thy1 is a cell surface glycophosphatidylinositol-linked glycoprotein with a molecular mass of 35 kDa and is an adhesion molecule of the immunoglobulin superfamily. In mice and rats, Thy1 is expressed in the brain, on thymocytes,

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T lymphocytes, fibroblasts, epidermal cells, and a small population of bone marrow cells. Thy1 is involved in T-cell activation and affects numerous nonimmunologic biological processes, such as cellular adhesion, neurite outgrowth, tumor growth, migration, and cell death.[15,16] In the liver, Thy1 expression was also detected in cell lines of human fetal hepatoblasts,[17] in a stem-like population (distinct from OCs), derived from adult human liver[18] and in hepatocellular carcinoma cell lines.[19]

In our previous work, we showed that in normal rat liver, several populations of cells express Thy1: circulating blood progenitor cells ($Thy1^+/CD45^+$), fibroblasts in the periportal region surrounding bile ducts and blood vessels, a small population of mesenchymal cells at the lobular interface, and cells in the liver lobule; these cells were not described previously.[20] Most $Thy1^+$ cells located at the lobular interface and in the parenchyma co-express desmin, but not Acta2 [alias α-smooth muscle actin (SMA)].[20] Thy1-expressing cells proliferate moderately after carbon tetrachloride acute injury, but in all models of OC-mediated liver regeneration, their number is increased substantially.[20] RT-PCR analyses showed that activated $Thy1^+$ cells do not express OC genes, but they express genes known to be expressed in mesenchymal stem cells, genes considered specific for activated stellate cells and myofibroblasts, and growth factors and cytokines that affect OC growth.[20] Subcloning of $Thy1^+$ cells from OC-activated livers yields $Thy1^+$ fibroblastic cells and a population of E-cadherin$^+$ mesenchymal-epithelial cells that express cytokeratins.[20]

In normal liver, Thy1-positive cells express desmin, but not Acta2, suggesting that they are not resident myofibroblasts or pericytes. Also, they are not portal fibroblasts, because they do not express CD39L1 and elastin.[21,22] In addition, it has been shown recently that glial fibrillary acidic protein (GFAP)—expressing activated hepatic stellate cells and myofibroblasts in thioacetamide-induced rat liver injury express desmin, Acta2, and vimentin, but they do not express Thy1.[23]

Mesenchymal stem cells recruited from bone marrow stroma and other adult tissues, including human liver, are of great potential significance for regenerative medicine. It was recognized recently that these cells exhibit enormous plasticity and, under appropriate stimuli, can differentiate not only into osteoblasts, chondrocytes, and adipocytes, but also into myocytes, neuronal cells, and hepatocyte-like cells, which underlines their importance.[24–27] The origin of the small $Thy1^+$ cells that expand quickly in the liver after injury and disappear when the insult is resolved has not been determined. One possibility is that they originate from bone marrow mesenchymal cells and are attracted to the injured liver.[28,29] Another possibility is that Thy1 cells are a subpopulation of hepatic mesenchymal cells that are activated simultaneously with OCs. If their origin is hepatic, they may constitute a mesenchymal progenitor-like population. Alternatively, these cells could be a product of epithelial-mesenchymal transition.[30]

Herein, we investigated further the nature of Thy1-expressing cells and characterized their mesenchymal-epithelial phenotype and progenitor-like characteristics in normal liver *in vivo* and *in vitro* and during OC-mediated liver regeneration. We also determined whether they have endogenous or bone marrow origin. Two models of OC-mediated liver regeneration were used: 2-AAF/PH and retrorsine (Rs) in conjunction with PH (Rs/PH). 2-AAF is metabolized in the liver, causing destruction of hepatocytes, formation of DNA adducts, and inability of residual hepatocytes to proliferate when PH is performed. In this model, massive hepatocyte proliferation begins after termination of the 2-AAF diet, and the liver returns to normal appearance within 14 days.[31] Rs is a member of the pyrrolizidine alkaloid family that blocks hepatocyte division and induces megalocytosis; its exact mechanism of action remains unclear. Rs exerts a strong and persistent cell cycle block ($G_1$, S, and/or late $G_2$). When PH is performed, liver regeneration remains incomplete, even after 16 weeks.[32]

## Materials and Methods

### Chemicals

All chemicals, including lipopolysaccharides (LPSs), were from Sigma (St. Louis, MO), unless otherwise stated.

### Cytokines and Mitogens

Recombinant rat interferon-gamma (IFN-γ); recombinant human IL-1α (carrier free); recombinant rat IL-1β; recombinant rat epidermal growth factor (EGF); recombinant human acidic fibroblast growth factor (FGF-1); recombinant rat acidic FGF-1; recombinant rat basic FGF-2; recombinant mouse platelet-derived growth factor BB (PDGF-BB); recombinant rat tumor necrosis factor α (TNF-α); recombinant rat IL-6; recombinant human hepatocyte growth factor (HGF); and recombinant human connective tissue growth factor (CTGF) were from ProSpec-Tany Technogene Ltd. (Ness Ziona, Israel).

Recombinant human PDGF-BB; recombinant rat IFN-γ; recombinant human IL-1α; recombinant rat IL-1β; recombinant human acidic FGF-1; recombinant human FGF-2; recombinant human TGF-β1; recombinant human FGF-4; recombinant human EGF; recombinant human TGF-α; recombinant mouse IL-6 were from PeproTech Inc. (Rocky Hill, NJ). Recombinant mouse IL-6 and recombinant human IL-1α were from BioLegend (San Diego, CA). Recombinant human HGF is from BioVision, Inc. (Milpitas, CA). Recombinant mouse HGF; recombinant human FGF-4; recombinant mouse PDGF-BB were from R&D Systems, Inc. (Minneapolis, MN).

### Antibodies

Primary antibodies are listed in Table 1. Anti-rabbit peroxidase—linked antibody was from Amersham Biosciences