DE GRUYTER OPEN

Acta Veterinaria-Beograd 2014, 64 (4), 397-412
UDK: 591.436
DOI: 10.2478/acve-2014-0038

Review article

MYOFIBROBLASTS IN NORMAL AND FIBROTIC LIVER IN DIFFERENT ANIMAL SPECIES

KUKOLJ Vladimir*

[1] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobodjenja 18, 11000 Belgrade , Serbia

(Received 10 October; Accepted 12 November 2014)

Myofibroblasts, cells sharing characteristics with fibroblasts and smooth muscle cells, may have a very heterogeneous origin. The myofibroblasts may be derived from a variety of sources including resident mesenchymal cells, epithelial to mesenchymal transition, as well as from circulating fibroblast-like cells called fibrocytes that are derived from bone-marrow stem cells, or derived from bone marrow precursors. In normal conditions, fibroblastic cells exhibit a low extracellular matrix production ability. After tissue injury, they become activated by cytokines locally released from inflammatory and resident cells to migrate into the damaged tissue and to synthesize extracellular matrix components. The investigation of cytoskeletal and cell surface markers showed a certain degree of heterogeneity of these cells. The reason for this is that markers these cells express to a large extent depend on the type of animal, age and stage of development of fibrosis. A better knowledge of the molecular mechanisms involved in the appearance of differentiated myofibroblasts in different pathological situations will be useful for understanding the development of fibrosis, its prevention and therapy.

Key words: fibrogenesis, immunohistochemistry, liver fibrosis, myofibroblasts.

INTRODUCTION

Hepatic fibrosis is the end result of chronic liver diseases induced by a variety of stimuli including persistent infection, toxins, viruses, bacteria, parasites and autoimmune reactions [1,2]. Hepatic fibrosis is defined as the excessive accumulation of extracellular matrix (ECM) proteins including collagen. As fibrotic liver diseases advance, progression from collagen bands to bridging fibrosis to frank cirrhosis occurs. Accordingly, cirrhosis can be defined as an advanced stage of fibrosis involving the formation of regenerative nodules of parenchyma surrounded and separated by fibrotic septa, a scenario also characterised by significant changes in hepatic angioarchitecture. Generally, the mechanism for the origin and development of liver fibrosis and cirrhosis is not fully known, and from the stand point of today's knowledge it includes: persistence of parenchymal damage with variable degree of

Corresponding author: e-mail: vkukolj@vet.bg.ac.rs

necrosis and apoptosis; presence of a heterogeneous inflammatory infiltrate including mononuclear cells and cells of the immune system; activation of different types of ECM-producing and myofibroblast-like cells with marked proliferative, synthetic and contractile features; and qualitative and quantitative changes of hepatic ECM, associated with limited or absent remodeling in the presence of a persistent attempt of hepatic regeneration. Many studies have clearly demonstrated that hepatic fibrosis is reversible in experimental rodent models (alcohol feeding, CCl4, or bile duct ligation). Upon removal of the etiological source of the chronic injury, regression of liver fibrosis is associated with decreased cytokines and ECM production, increased collagenase activity, disappearance of myofibroblasts (MFs) population and dissolution of the fibrous scar. Only recently has the fate of these MFs been revealed. The previous concept was that the MFs undergo apoptosis on the basis of documented senescence during reversal of fibrosis. Some authors have used genetic marking to demonstrate an alternative pathway in which MFs revert to a quiescent-like phenotype in CCl4-induced liver injury. Genetic marking of MFs enabled the quantitative mapping of the fate of these cells in experimental models of fibrosis and its reversal [3,4].

In the progression of chronic liver entry to fibrosis, all hepatic cells undergo specific changes. The hepatocytes are injured and they undergo apoptosis. The sinusoidal endothelial cells undergo a loss of fanestrae that is termed acapillarization of the sinusoids. The resident macrophages in the liver, the Kupffer cells, activate and produce a variety of chemokines and cytokines. Lymphocytes infiltrate the injured liver and contribute to the inflammation. Finally, the quiescent stellate cells are activated to produce extracellular matrix proteins. Development of liver fibrosis entails major alterations in both quantity and quality of hepatic ECM. In advanced stages, the liver contains approximately 6 times more ECM than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Accumulation of ECM results from both increased synthesis and decreased degradation. Decreased activity of ECM-removing matrix metalloproteinases MMPs is mainly due to an overexpression of their specific inhibitors – tissue inhibitor of matrix metalloproteinases. Hepatic stellate cells (HSCs) are the main ECM-producing cells in the injured liver. There is overwhelming evidence that activated MFs are the major source of the components of ECM [5]. The cells responsible for fibrosis in all chronic liver diseases appear to be the activated MFs.

MFs are the main effectors of fibrosis in all tissues. They also make a major contribution to other aspects of the wound healing response, including regeneration, inflammation, and angiogenesis, normal tissue repair after acute injury and to the stromal reaction in tumors [6]. They combine phenotypic features of fibroblasts, such as the production of ECM, with the contractile functions of smooth muscle cells involved in tissue architecture distortion. During progression of chronic liver fibrosis, resident hepatic cells undergo specific changes and begin to produce extracellular matrix proteins. In normal conditions, fibroblastic cells exhibit few or no actin-associated cell-cell and cell-matrix contacts and little ECM production. After tissue injury, they become activated

to migrate into the damaged tissue and to synthesize ECM components by cytokines locally released from inflammatory and resident cells [7-9].

GENERAL CHARACTERISTICS OF MFS

The role of MFs in different pathological processes is well established. Recent reports of several studies performed on human patients indicate that myofibroblasts have an important role in the synthesis of ECM components and development liver fibrosis and cirrhosis. Studies of primary cultures of MFs and in mouse models of hepatic fibrosis have revealed several common pathophysiological mechanisms such as oxidative stress, increased transforming growth factor beta, hepatocyte death and chronic inflammation.

Liver fibrogenesis is sustained by populations of highly proliferative, pro-fibrogenic and contractile MFs that, according to current literature, originate by a process of activation involving perisinusoidal HSC, portal fibroblasts and even bone marrow-derived MFs. According to the morphological and functional characteristics of MFs, these cells are between fibroblasts and smooth-muscle cells. MFs are capable of expressing a variety of cytoskeletal proteins which are used as markers of their differentiation. MFs contain microfilament bundles and/or stress fibers in the cytoplasm which play an important role in the mechanism of contraction that is similar to the mechanism of contraction of smooth-muscle cells [10-12].

Three different MFs-like cells have been described in rats and humans based on the location and immunohistochemical profile. These comprise: a) portal or septal MFs, present in the portal areas, or in newly formed fibrous septa, and for the most part come from the portal fibroblasts, b) interface MFs, present at the interface between parenchyma and stroma of the portal areas or newly formed fibrous septa and according to their antigen profile, probably originate from activated hepatic stellate cells, and c) the perisinusoidally located HSCs originating from quiet inactive HSCs [13-17].

ORIGIN OF MFS

During liver development, the septum transversum-derived mesothelium, which signals to induction of hepatogenesis from the foregut endoderm, gives rise to sinusoidal pericytes, called hepatic stellate cells (HSCs), and perivascular mesenchymal cells, including portal fibroblasts, smooth muscle cells and fibroblasts around the central veins. All these cells therefore have a common mesodermal origin, different from that of sinusoidal endothelial cells, Kupffer cells and hepatoblasts. It is now more than 35 years since the initial demonstration by Hans Popper and coworkers that transitional cells with the morphologic characteristics of vitamin A-containing cells (i.e. HSCs) and fibroblasts overproduce fibrillar collagen in rats with carbon tetrachloride-induced

liver injury. The paradigm of HSCs activation giving rise to MFs has since dominated the focus of research on liver fibrosis. HSC was the first major cell type in the liver to be identified as a prominent source of collagen production in the injured liver [6,17].

Currently, the origin of the acitvated MFs is unresolved, and several cells potentially can fulfill this role. The MFs may be derived from a variety of sources including resident mesenchymal cells, epithelial to mesenchymal transition, as well as from circulating fibroblast-like cells called fibrocytes that are derived from bone-marrow stem cells, or derived from bone marrow precursors [2,7,18]. Also, MFs derived from small portal vessels proliferate around biliary tracts in cholestasis-induced liver fibrosis to initiate collagen deposition [19-21]. The bone marrow-derived fibrocytes, or circulating mesenchymal cells, can migrate through the injured liver and become MFs to participate in the fibrotic process. These circulating mesenchymal stem cell progenitors have a fibroblast/myofibroblast-like phenotype and they express CD34, CD45 and type I collagen [2,22]. Alternatively, there is a limited amount of evidence that hepatocytes, cholangiocytes, or even endothelial cells may undergo a transition to mesenhymal cells to become activated MFs. For hepatocytes and cholangiocytes, this process is called the epithelial-to-mesenhymal transition or EMT. Finally, the resident cells in the liver may be activated to become MFs [23].

Portal fibroblasts found in the connective tissue around blood vessels and bile ducts, are the dominant source of MFs (mainly portal and septal) in the development of fibrosis induced by bile duct ligation. Portal fibroblasts are a heterogeneous population and represent one of several fibroblast populations in the liver. The term "portal fibroblast" refers to any fibroblast in the portal region, and the term "portal MFs" to any MFs that originates in the portal area and is not derived from HSCs. In addition to portal fibroblasts, in chronic viral hepatitis fibrogenic cells located on the interface between the portal and parenchymal area are the main precursors of MFs. The second layer fibroblasts located around the central veins are the most important source of MFs in the development of alcoholic liver fibrosis and cirrhosis [4,8,10,13,16,24,25].

Each of the mentioned sources of activated MFs participate with varying percentage in the synthesis of the ECM depending on the organ in which fibrosis is developed. HSCs have the most important role in ECM synthesis during the development of fibrosis in liver. These cells contribute 70-80% of all matrix-producing cells in the liver. The contribution of MFs in the ECM component synthesis depends on their origin. It was proven that MFs derived from the local fibroblasts constitute 4-6% of all collagen-producing cells, and MFs derived from bone marrow fibroblasts / fibrocytes are 8-12% of all collagen-producing cells. The percentage contribution of MFs created by the EMT is not known [10,26].

Moreover, immunohistochemical studies have shown that, in fibrotic human or rat liver, portal and septal myofibroblasts have expression profiles different from those of interface myofibroblasts or perisinusoidally located HSCs, suggesting that at least two subpopulations of myofibroblasts — HSC-derived myofibroblasts (HSC-MFs)

and portal mesenchymal cell-derived myofibroblasts (PMFs) — populate the injured liver [6].

Activation of portal fibroblasts, and their transformation in MFs, is usually caused by ischemic conditions and biliary obstructive cholestatic diseases. Due to the very similar antigen-expressing features it is believed that the portal and septal MFs have an identical origin [16,21,27,28].

Some studies have confirmed that in the chronic liver damage profibrogenic MFs (mainly interface MFs and portal MFs) are derived from bone marrow mesenchymal stem cells and circulating fibroblasts [25,28]. In carbon tetrachloride induced fibrosis, 33% of liver MFs expresses markers (CD11b, MHCII i F4/80) which are typical for bone marrow derived cells [10,25,26].

Epithelial-to-mesencymal transition (EMT) is the process of transdifferentiation of differentiated epithelial cells followed by phenotypic changes and transition into differentiated mesenchymal cells - fibroblasts and MFs. Basically, this process means reprogramming of gene expression, modification of transcription processes, architecture, adhesion and the ability to migrate. Several steps are critical for EMT: 1) disruption of local basement membrane; 2) loss of epithelial cell adhesion; 3) reprogramming of signaling machinery and de novo synthesis of α-smooth muscle actin (α-SMA); and 4) rearrangement of cytoskeletal proteins and transmigration of epithelial cells through the basement membrane into the interstitial space [26,29,30].

Biliary epithel cells expression of S100A4, an early fibroblast lineage marker established as a robust marker of EMT, as well as vimentin, α-SMA and pSmad 2/3 were identified in liver tissue from patients with primary biliary cirrhosis. This result suggests that EMT of biliary epithelial cells is occurring and that this process is driven by TGF-β [31].

HSCS AS A MAJOR SOURCE OF MFS

HSCs are a heterogeneous group of cells that are functionally and anatomically similar, but they differ in the expression of cytoskeletal filaments, the retinoid contents and the potential for the formation of ECM. HSCs pathologists have known for more than a century, ever since the moment when they were in 1876 described by von Kupfer. HSCs have been described in several mammalian species, including humans, but also in lower vertebrates, such as fish. Several decades ago, the role of the HSC in the deposition of lipo-soluble vitamin A in its stable form was described. These cells were previously called Ito cells, lipocytes or perisinusoidal cells. HSCs reside in the space of Disse and in normal conditions have the function in vitamine A storage in the form of retinyl esters. Following chronic liver injury, HSCs proliferate, lose their vitamin A and undergo a major phenotypical transformation to α-SMA positive MFs which produce a wide variety of collagenous and non-collagenous ECM components [32-35]. HSCs are the primary source of ECM in the normal and fibrotic liver. Today there

is substantial evidence that HSCs are the most important source for the production of collagen and noncollagenous ECM proteins. The discovery of the role of HSCs in the development of liver fibrosis is directed towards testing the evidence of their importance for prognosis and possible treatment of liver fibrosis [36-39].

The nature of HSCs, which are traditionally grouped into cells of mesenchymal origin because of their morphological appearance, as well as expression of molecules such as vimentin, desmin and α-SMA, is brought under question, because numerous studies clearly demonstrate the expression of neural and neuroendocrine markers, such as the glial fibrilar acid protein (GFAP), nestin, synaptophysin and neurotrophin receptor suggesting that HSCs originate from the neural crest [13,40]. Some authors assume the possible epithelial origin of these cells and the potential EMT that occurs during their activation [36].

In normal human liver HSCs accounted for about 1.4% of the total volume of the liver, and 15% (according to some authors 5-8%) of the total number of cells located in the liver. The number of HSCs is 3.6 to 6 HSCs per 100 hepatocytes or in a ratio of 1 HSC to 20 hepatocytes [10,41]. HSCs have a stellar appearance due to their dendritic cytoplasmic extensions that are partly linked to neighboring endothelial cells and extending between hepatocytes and communicate with other cytoplasmic extensions of HSCs [36,40,41]. HSCs consist of a body and a number of dendritic extensions. The increase in contractility, loss of normal storage capacity for retinoids, increasing the amount of granular endoplasmic reticulum, changes in the organization of the cytoskeleton and cell morphology as well as acquiring the ability to synthesize ECM are the most important phenotypic characteristics of activated HSCs. Loss of fat droplets is considered to be the earliest morphological change indicating the activation of HSCs. Loss of fat droplets takes place after HSCs activation of hydrolysis of retinyl esters needed in order for retinol to leave the cell. HSCs showing properties of peaceful and activated cells are often referred to as transitional cell [19,37,40-43].

ACTIVATION OF MFS

After hepatic injury, MFs activation and migration to damaged tissue, as well as synthesis of ECM components take place. Cytokines that are locally released from inflammatory and resident cells of the liver have a key role in the activation process as well as in MFs phenotype changes. In addition to cytokines, an essential role in the phenotypic change has the microenvironment in which the MFs are.

It is helpful to understand the pathophysiology of hepatic fibrosis based on the framework of hepatic stellate cell activation. Activation consists of two major phases, initiation (also called a “preinflammatory stage”) and perpetuation, followed by a resolution phase when liver injury resolves. Initiation refers to early changes in gene expression and phenotype that render the cells responsive to other cytokines and stimuli. Initiation results mostly from paracrine stimulation, primarily due to

changes in the surrounding extracellular matrix, as well as exposure to lipid peroxides and products of damaged hepatocytes [19,43-45]. Perpetuation results from the effects of these stimuli on maintaining the activated phenotype and generating fibrosis. Perpetuation involves autocrine as well as paracrine loops. It is comprised of several discrete responses including proliferation, contractility, fibrogenesis, matrix degradation, retinoid loss, and inflammatory cell infiltration [40,42]. Resolution of fibrosis refers to pathways that either drive the stellate cells to apoptosis, or contribute to their reversion to a more quiescent phenotype.

MFs are activated by a variety of mechanisms, including paracrine signals derived from lymphocytes and macrophages, autocrine factors secreted by MFs, and pathogen-associated molecular patterns (PAMPS) produced by pathogenic organisms that interact with pattern recognition receptors (i.e. TLRs) on fibroblasts. The interaction between PAMPs and PRRs serves as a first line of defence during infection and activates numerous proinflammatory cytokine and chemokine responses. Thus, inhibiting TLR signalling might represent a novel approach to treat fibrotic disease. Cytokines (IL-13, IL-21, TGF-β1), chemokines (MCP-1, MIP-1β), angiogenic factors (VEGF), growth factors (PDGF), peroxisome proliferator-activated receptors (PPARs), acute phase proteins (SAP), caspases, and components of the renin–angiotensin–aldosterone system (ANG II) have been identified as important regulators of fibrosis and are being investigated as potential targets of antifibrotic drugs [2].

Although each of the mentioned cells releases mediators which have different effects on cells in the production of ECM components, undoubtedly the greatest number of papers highlights the role of PDGF and TGF-β. PDGF, mainly produced by Kupffer cells, is the predominant mitogen for activated HSCs that stimulates their proliferation, while TGF-β is considered the most important cytokine that stimulates HSCs to fibrogenesis [19,44,46].

Various cytokines and growth factors have a role in the process of MFs activation. Among these, soluble factors stimulate fibrogenic cell activation, especially transforming growth factor-β1 (TGF-β1), a potent inducer of myofibroblastic differentiation. Beyond a specific effect on the induction of α-smooth muscle expression, TGF-β1 also promotes the deposition of large amounts of extracellular matrix [47].

After tissue injury, fibroblasts differentiate into contractile and secretory myofibroblasts that contribute to tissue repair during wound healing, but that can severely impair organ function when contraction and ECM protein secretion becomes excessive. At least three local events are needed to generate α-SMA-positive differentiated myofibroblasts: 1) accumulation of biologically active TGF-β1, 2) the presence of specialized ECM proteins like the ED-A splice variant of fibronectin, and 3) high extracellular stress, arising from the mechanical properties of the ECM and cell remodeling activity [8]. Differentiation of fibroblasts into MFs can be understood as a two-step process: 1) to re-populate damaged tissues, fibroblasts acquire a migratory phenotype by de novo developing contractile bundles. These *in vivo* stress fibers are

first composed of cytoplasmic actins and generate comparably small traction forces. Most authors use the term "proto-myofibroblast" to discriminate such activated fibroblasts from quiescent fibroblasts that are devoid of any contractile apparatus in most intact tissues. This first phenotypic change occurs in response to changes in the composition, organization, and mechanical property of ECM and to cytokines locally released by inflammatory and resident cells [48]. 2) With increasing stress in the ECM resulting from their own remodeling activity, proto-myofibroblasts further develop into "differentiated myofibroblasts" by neo-expressing α-SMA, the most widely used myofibroblast marker [47,49,50]. Expression of α-SMA is precisely controlled by the joint action of growth factors like TGF-β1, of specialized ECM proteins like fibronectin, splice variant ED-A fibronectin, as well as the mechanical microenvironment. Incorporation of α-SMA into stress fibers significantly augments the contractile activity of fibroblastic cells and hallmarks the contraction phase of connective tissue remodeling [8,51].

IMMUNOHISTOCHEMICAL CHARACTERISTIC OF DORMANT AND ACTIVATED MFS

Quiescent HSCs express markers that are characteristic of adipocytes (PPARγ, SREBP-1c, and leptin), while activated HSCs express myogenic markers (α smooth muscle actin, c-myb, and myocyte enhancer factor–2).

The most widely used and accessible marker of these cells is the de novo expression of α-SMA, although this is not an absolute requirement for the identification of a cell as a MF. Other markers of MFs (endosialin, P311, integrin α11β1, osteopontin, periostin) have been proposed, but all were identified in specific conditions, and it remains unclear whether they could serve as general markers [49].

About the immunophenotypic characteristics of dormant and activated MFs there is no unique data. The investigation of cytoskeletal and cell surface markers showed a certain degree of heterogeneity of these cells. The reason for this is that markers that these cells express on a large extent depend on the type of animal, age and stage of development of fibrosis [52]. There is information that the environment in which HSCs are located can determine their phenotype [53].

The degree of activation of MFs and the development of fibrosis depend on the age of the animals. Young rats with a ligated bile duct rapidly increased the number of activated MFs and portal fibrosis developed faster than in adult rats [54]. The intensity of desmin expression in MFs after ligation is higher in young rats than in adults. In contrast to desmin, α-SMA expression between the two groups showed no statistical significance [52].

Investigation of HSCs in fish revealed the presence of beta-tubulin, α-SMA, smooth muscle type myosin, desmin and cytokeratin but not vimentin or glial fibrillar

acidic protein [55]. Inactive HSCs in broilers express vimentin, desmin, GFAP and cytokeratine [56].

In normal rat liver HSCs are situated in the perisinusoidal spaces and they are immunopositive to GFAP, desmin, vimentin, sinemin and V-CAM-1. Desmin and vimentin positive MFs in the rat liver have long cytoplasmatic extensions. Desmin expressing cells are visible in the portal spaces in arterial and venous blood vessels wall (portal fibroblasts). In normal liver parenchyma of rats α-SMA, β-tubulin, fibulin-2, N-CAM and cytokeratine expression has not been proven [57,58]. Activation of quiet HSCs and their consequent differentiation in MFs in humans and rats accompanied by expression of α-SMA, an isoform of actin, which is only present in smooth-muscle cells of blood vessels in normal or damaged liver [59].

Contrary to rat liver, in normal canine livers perisinusoidal HSCs react positively to the α-SMA antibody (Fig. 1a) and are poorly positive to desmin (Fig. 1b), diffusely, through the entire liver parenchyma. In the cytoplasm of these cells large lipid vacuoles with a dislocated nucleus to the periphery are revealed. The positive α-SMA reaction of smooth muscle cells of terminal and sublobular venous blood vessels are observed. In the portal triads, a positive reaction is observed in the arterial tunica media, and a slight positivity is observed in the walls of the portal veins. In normal canine liver HSCs are negative for vimentin and weakly positive for desmin, while the portal fibroblasts stain positive for vimentin and weakly positive for desmin [14,60].

In feline normal liver HSCs show a positive reaction with antibodies against α-SMA (Fig. 1c) and desmin (Fig. 1d). Moderately discontinuous reactivity to α-SMA is present in the spaces of Disse, randomly distributed around the bile ducts and blood vessels and under the Glisson's capsule. Mild positive reaction to desmin is observed in portal blood vessels and some perisinusoidal cells [61].

HSCs in normal porcine liver have long cytoplasmatic extensions, but they are shorter then cytoplasmatic extensions in rat HSCs. HSCs are desmin, vimentin and sinemin positive, but α-SMA negative in the liver of healthy swine. The number and size of the cytoplasmic vacuoles of porcine HSCs are constant irrespective of age and hepatic lipid contents [58,62].

In normal bovine liver HSCs are situated in the perisinusoidal spaces and have long cytoplasmatic extensions. In the citoplasm small vacuoles about 5 to 7μm in diameter are visible. Bovine HSCs are positive for desmin, but negative for α-SMA antibodies [62].

In some wild animals, like fallow deer, hepatic MFs are similar to those described in domestic ruminants and in healthy animals are α-SMA (Fig. 1e) and desmin (Fig. 1f) positive [63].

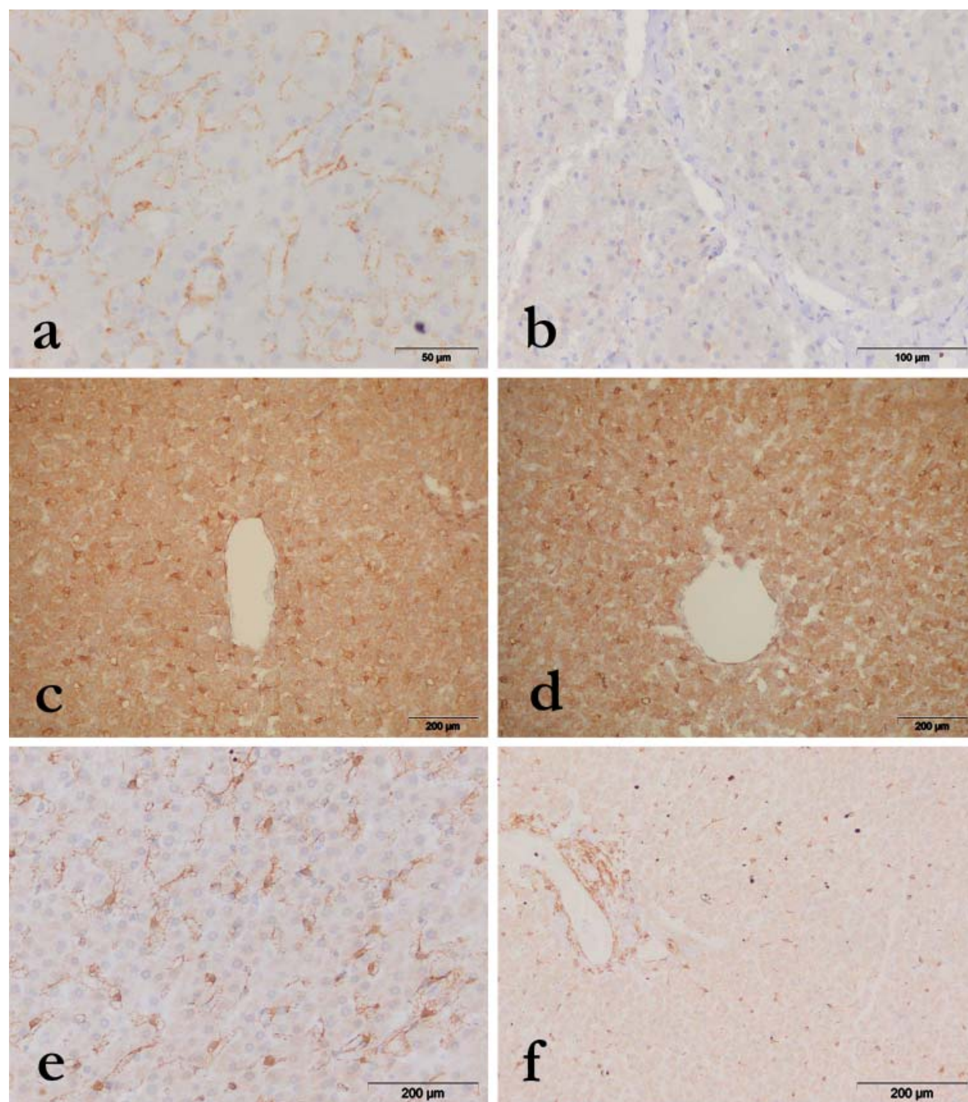

Figure 1. Imunophenotipic characteristics of MFs in normal canine (a,b), feline (c,d) and fallow deer (e,f) liver. **a)** α-SMA immunopositive MFs in normal canine liver, LSAB2, α-SMA; **b)** Desmin expression in normal canine liver, LSAB2, desmin; **c)** α-SMA expression in normal feline liver, LSAB2, α-SMA; **d)** Cells immunopositive to desmin in normal feline liver, LSAB2, desmin; **e)** α-SMA immunopositivity in normal fallow deer liver, LSAB2, α-SMA; **f)** Desmin expression in normal fallow deer liver, LSAB2, desmin.

In the early stage of fibrosis in the liver of rats a small amount of connective tissue and thin connective-tissue septa are present. The presence of cells that are positive for α-SMA, desmin and N-CAM can be seen in the connective-tissue septa (portal and/or septal MFs). Cells that are positive for desmin and GFAP are present at the interface between septa and hepatic parenchyma (interface MFs), as well as in the

parenchyma (HSCs) [57]. In advanced stages of rat liver fibrosis mainly activated HSCs are immunoreactive for GFAP and desmin. HSCs in fibrotic rat liver show weak immunopositivity to α-SMA, N-CAM, synaptophysin, neurotrophins, neurotrophin receptors and alpha B-crystallin. Interface MFs at the septal/parenchymal interface showed an expression profile, intermediate between the profiles of HSCs and portal/septal MFs. They are immunoreactive to desmin, GFAP, α-SMA, N-CAM and alfa B-crystallin. If centrolobular fibrosis in rats is induced by bile duct ligation, the reaction of the second layer of cells around the central vein is observed. These cells express α-SMA, GFAP and alfa B-crystallin, a slightly weaker dezmin and N-CAM [13,64].

In canine liver with fibrosis, immunopositivity to α-SMA (Fig. 2a), desmin (Fig. 2b), and weakly to vimentin is observed on perisinusoidal HSCs and their long extensions, in the form of deposits are of a beaded appearance. Livers of dogs with moderate or severe fibrosis showed numerous α-SMA, desmin and vimentin positive cells in fibrous septs and the stroma that surrounded the regenerative lobules (portal/septal MFs). With the increase in the degree of fibrosis, the degree of expression of α-SMA also increased, both in the HSCs and in other localities [60,65,66].

In the mildest forms of feline liver fibrosis α-SMA (Fig. 2c) and desmin (Fig. 2d) is expressed in HSCs and portal/septal MFs. In the liver with moderate to severe fibrosis numerous α-SMA positive cells, presumable MFs, are present in the fibrous septa, and stroma surrounding regenerative nodules. In feline liver with periportal and septal fibrosis α-SMA is detected in round and spindle-shaped cells around bile ducts and blood vessel walls. Scarce interface stellate cells and perisinusoidal cells with processes showed an intensive positive reaction to α-SMA. In fibrous septa a positive reaction to desmin was detected in MFs and blood vessels, as well as in some HSCs at the periphery of the lobuli [61].

MFs in bovine liver due to Fasciola infection are spindle-shaped and positive for vimentin, desmin and α-SMA. These cells are increased in the peribiliary connective tissue, although the desmin-positive cells are fewer [67]. Similarly to those, HSCs, portal/septal MFs and interface MFs are positive to α-SMA (Fig. 2e) and desmin (Fig. 2f) in the liver of fallow deer due to *Fascioloides magna* infection [63].

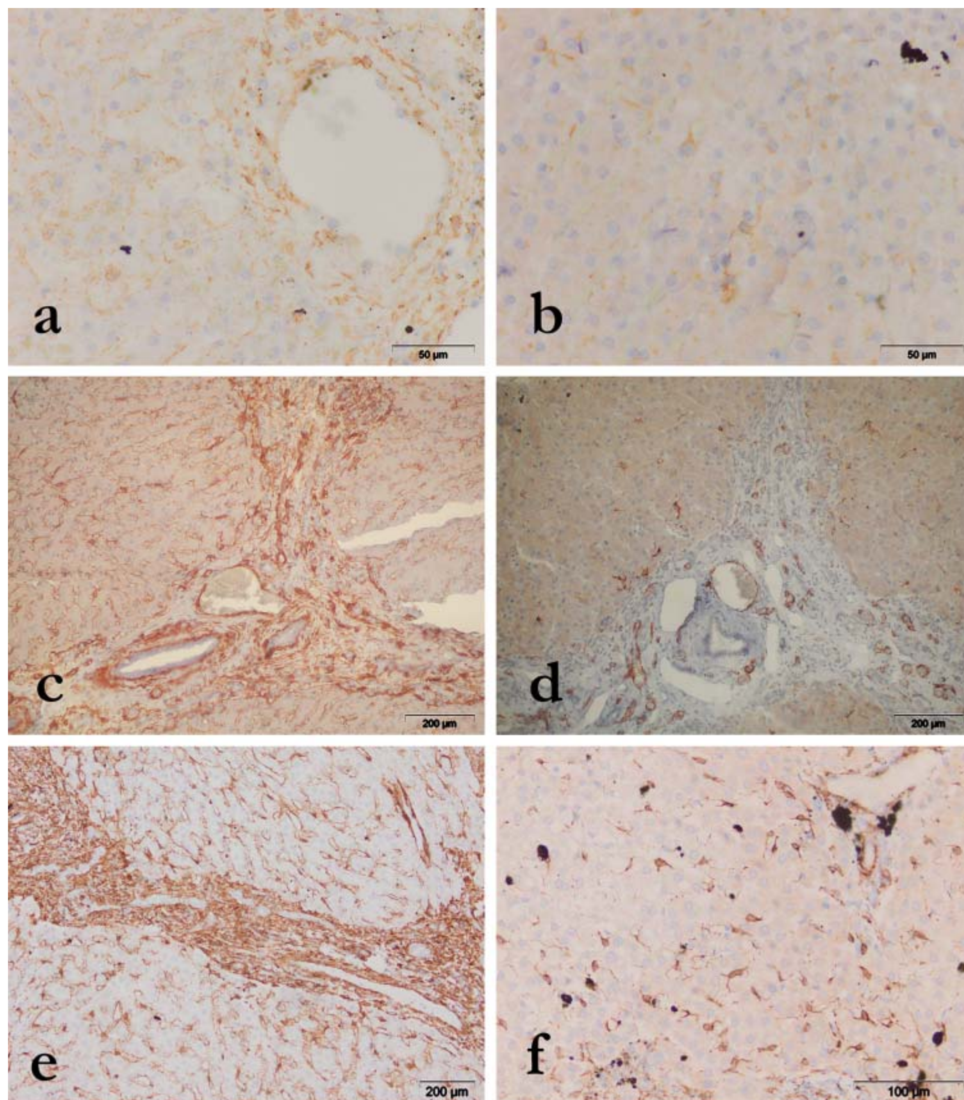

Figure 2. Imunophenotipic characeristics of MFs in fibrotic canine (a,b), feline (c,d) and fallow deer (e,f) liver. **a)** α-SMA expression on MFs in fibrotic canine liver, LSAB2, α-SMA; **b)** Desmin expression in fibrotic canine liver, LSAB2, desmin; **c)** α-SMA immunopositivity in feline fibrotic liver, LSAB2, α-SMA; **d)** Cells immunopositive to desmin in fibrotic feline liver, LSAB2, desmin; **e)** Distribution of α-SMA positive cells in fibrotic fallow deer liver, LSAB2, α-SMA; **f)** Desmin expression in fibrotic fallow deer liver, LSAB2, desmin.

Acknowledgements

I appreciate Prof. Dr. Sanja Aleksić-Kovačević for critically reading the manuscript.

REFERENCES

1. Anthony B, Allen JT, Li YS, McManus DP: Hepatic stellate cells and parasite-induced liver fibrosis. Parasit Vectors 2010, 3(1):60, doi: 10.1186/1756-3305-3-60.
2. Wynn TA: Cellular and molecular mechanisms of fibrosis. J Pathol 2008, 214(2):199-210.
3. Jiroutova A, Peterova E, Bittnerova L, Slavkovsky R, Cevelova P, Rezacova M, Cerman J, Micuda S, Kanta J: Collagenolytic potential of rat liver myofibroblasts. Physiol Res 2013, 62(1):15-25.
4. Parola M, Pinzani M: Hepatic wound repair. Fibrogenesis Tissue Repair 2009, 2(1):4, doi: 10.1186/1755-1536-2-4.
5. Benyon RC, Iredale JP: Is liver fibrosis reversible? Gut 2000, 46(4):443-446.
6. Lemoinne S, Cadoret A, El Mourabit H, Thabut D, Housset C: Origins and functions of liver myofibroblasts. Biochim Biophys Acta 2013, 1832(7):948-954.
7. Brenner DA: Molecular pathogenesis of liver fibrosis. Trans Am Clin Climatol Assoc 2009, 120:361-368.
8. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G: The myofibroblast: one function, multiple origins. Am J Pathol 2007, 170(6):1807-1816.
9. Kendall RT, Feghali-Bostwick CA: Fibroblasts in fibrosis: novel roles and mediators. Front Pharmacol 2014, 5:123, doi: 10.3389/fphar.2014.00123.
10. Guyot C, Lepreux S, Combe C, Doudnikoff E, Bioulac-Sage P, Balabaud C, Desmouliere A: Hepatic fibrosis and cirrhosis: the (myo)fibroblastic cell subpopulations involved. Int J Biochem Cell Biol 2006, 38(2):135-151.
11. Honda E, Park AM, Yoshida K, Tabuchi M, Munakata H: Myofibroblasts: Biochemical and proteomic approaches to fibrosis. Tohoku J Exp Med 2013, 230(2):67-73.
12. Xu J, Liu X, Koyama Y, Wang P, Lan T, Kim IG, Kim IH, Ma HY, Kisseleva T: The types of hepatic myofibroblasts contributing to liver fibrosis of different etiologies. Front Pharmacol 2014, 5:167, doi: 10.3389/fphar.2014.00167.
13. Cassiman D, Libbrecht L, Desmet V, Denef C, Roskams T: Hepatic stellate cell/myofibroblast subpopulations in fibrotic human and rat livers. J Hepatol 2002, 36(2):200-209.
14. Ijzer J, Roskams T, Molenbeek RF, Ultee T, Penning LC, Rothuizen J, van den Ingh TS: Morphological characterisation of portal myofibroblasts and hepatic stellate cells in the normal dog liver. Comp Hepatol 2006, 5:7, doi:10.1186/1476-5926-5-7.
15. Kisseleva T, Cong M, Paik Y, Scholten D, Jiang C, Benner C, Iwaisako K, Moore-Morris T, Scott B, Tsukamoto H, Evans SM, Dillmann W, Glass CK, Brenner DA: Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc Natl Acad Sci U S A 2012, 109(24):9448-9453.
16. Parola M, Marra F, Pinzani M: Myofibroblast - like cells and liver fibrogenesis: Emerging concepts in a rapidly moving scenario. Mol Aspects Med 2008, 29(1-2):58-66.
17. Ramadori G, Saile B: Portal tract fibrogenesis in the liver. Lab Invest 2004, 84(2):153-159.
18. Iwaisako K, Jiang C, Zhang M, Cong M, Moore-Morris TJ, Park TJ, Liu X, Xu J, Wang P, Paik YH et al: Origin of myofibroblasts in the fibrotic liver in mice. Proc Natl Acad Sci U S A 2014, 111(32):E3297-3305.
19. Bataller R, Brenner DA: Liver fibrosis. J Clin Invest 2005, 115(2):209-218.
20. Kinnman N, Housset C: Peribiliary myofibroblasts in biliary type liver fibrosis. Front Biosci 2002, 7:d496-503.

21. Tuchweber B, Desmouliere A, Bochaton-Piallat ML, Rubbia-Brandt L, Gabbiani G: Proliferation and phenotypic modulation of portal fibroblasts in the early stages of cholestatic fibrosis in the rat. Lab Invest 1996, 74(1):265-278.
22. Iwaisako K, Brenner DA, Kisseleva T: What's new in liver fibrosis? The origin of myofibroblasts in liver fibrosis. J Gastroenterol Hepatol 2012, 27 Suppl 2:65-68.
23. Brenner DA, Kisseleva T, Scholten D, Paik YH, Iwaisako K, Inokuchi S, Schnabl B, Seki E, De Minicis S, Oesterreicher C, Taura K: Origin of myofibroblasts in liver fibrosis. Fibrogenesis Tissue Repair 2012, 5 Suppl 1:S17.
24. Fausther M, Lavoie EG, Dranoff JA: Contribution of Myofibroblasts of Different Origins to Liver Fibrosis. Curr Pathobiol Rep 2013, 1(3):225-230.
25. Forbes SJ, Parola M: Liver fibrogenic cells. Best Pract Res Clin Gastroenterol 2011, 25(2):207-217.
26. Kisseleva T, Brenner DA: Mechanisms of fibrogenesis. Exp Biol Med (Maywood) 2008, 233(2):109-122.
27. Novo E, Cannito S, Paternostro C, Bocca C, Miglietta A, Parola M: Cellular and molecular mechanisms in liver fibrogenesis. Arch Biochem Biophys 2014, 548C:20-37.
28. 28. Novo E, di Bonzo LV, Cannito S, Colombatto S, Parola M: Hepatic myofibroblasts: a heterogeneous population of multifunctional cells in liver fibrogenesis. Int J Biochem Cell Biol 2009, 41(11):2089-2093.
29. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG: Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest 2002, 110(3):341-350.
30. Kalluri R, Neilson EG: Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 2003, 112(12):1776-1784.
31. Robertson H, Kirby JA, Yip WW, Jones DE, Burt AD: Biliary epithelial-mesenchymal transition in posttransplantation recurrence of primary biliary cirrhosis. Hepatology 2007, 45(4):977-981.
32. Hautekeete ML, Geerts A: The hepatic stellate (Ito) cell: its role in human liver disease. Virchows Arch 1997, 430(3):195-207.
33. Jing XY, Yang XF, Qing K, Ou-yang Y: Roles of the lipid metabolism in hepatic stellate cells activation big up tri, open. Chin Med Sci J 2013, 28(4):233-236.
34. Mallat A, Lotersztajn S: Reversion of hepatic stellate cell to a quiescent phenotype: From myth to reality? J Hepatol 2013, 59(2):383-386.
35. Senoo H: Structure and function of hepatic stellate cells. Med Electron Microsc 2004, 37(1):3-15.
36. Atzori L, Poli G, Perra A: Hepatic stellate cell: a star cell in the liver. Int J Biochem Cell Biol 2009, 41(8-9):1639-1642.
37. Burt AD: Pathobiology of hepatic stellate cells. J Gastroenterol 1999, 34(3):299-304.
38. Friedman SL, Rockey DC, McGuire RF, Maher JJ, Boyles JK, Yamasaki G: Isolated hepatic lipocytes and Kupffer cells from normal human liver: morphological and functional characteristics in primary culture. Hepatology 1992, 15(2):234-243.
39. Reeves HL, Friedman SL: Activation of hepatic stellate cells--a key issue in liver fibrosis. Front Biosci 2002, 7:d808-826.
40. Friedman SL: Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000, 275(4):2247-2250.
41. Moreira RK: Hepatic stellate cells and liver fibrosis. Arch Pathol Lab Med 2007, 131(11):1728-1734.

42. Friedman SL: Hepatic fibrosis -- overview. Toxicology 2008, 254(3):120-129.
43. Safadi R, Friedman SL: Hepatic fibrosis--role of hepatic stellate cell activation. MedGenMed 2002, 4(3):27.
44. Kramann R, DiRocco DP, Humphreys BD: Understanding the origin, activation and regulation of matrix-producing myofibroblasts for treatment of fibrotic disease. J Pathol 2013, 231(3):273-289.
45. Liu X, Xu J, Brenner DA, Kisseleva T: Reversibility of Liver Fibrosis and Inactivation of Fibrogenic Myofibroblasts. Curr Pathobiol Rep 2013, 1(3):209-214.
46. Gressner OA, Gao C: Monitoring fibrogenic progression in the liver. Clin Chim Acta 2014, 433C:111-122.
47. Micallef L, Vedrenne N, Billet F, Coulomb B, Darby IA, Desmouliere A: The myofibroblast, multiple origins for major roles in normal and pathological tissue repair. Fibrogenesis Tissue Repair 2012, 5 Suppl 1:S5.
48. Jiang JX, Torok NJ: Liver Injury and the Activation of the Hepatic Myofibroblasts. Curr Pathobiol Rep 2013, 1(3):215-223.
49. Rao KB, Malathi N, Narashiman S, Rajan ST: Evaluation of myofibroblasts by expression of alpha smooth muscle actin: a marker in fibrosis, dysplasia and carcinoma. J Clin Diagn Res 2014, 8(4):ZC14-17.
50. Rockey DC, Weymouth N, Shi Z: Smooth muscle alpha actin (Acta2) and myofibroblast function during hepatic wound healing. PLoS One 2013, 8(10):e77166, doi: 10.1371/journal.pone.0077166.
51. Lepreux S, Dubuisson L, Le Bail B, Desmouliere A, Balabaud C, Bioulac-Sage P: Can hepatic stellate cells express alpha-smooth muscle actin in normal human liver? Liver 2001, 21(4):293-294.
52. Gibelli NE, Tannuri U, Mello ES: Immunohistochemical studies of stellate cells in experimental cholestasis in newborn and adult rats. Clinics (Sao Paulo) 2008, 63(5):689-694.
53. Wells RG: The role of matrix stiffness in hepatic stellate cell activation and liver fibrosis. J Clin Gastroenterol 2005, 39(4 Suppl 2):S158-161.
54. Dranoff JA, Wells RG: Portal fibroblasts: Underappreciated mediators of biliary fibrosis. Hepatology 2010, 51(4):1438-1444.
55. Senda T, Nomura R: The expression of cytokeratin in hepatic stellate cells of the cod. Arch Histol Cytol 2003, 66(5):437-444.
56. Handharyani E, Ochiai K, Iwata N, Umemura T: Immunohistochemical and ultrastructural study of ito cells (fat-storing cells) in response to extrahepatic bile duct ligation in broiler chickens. J Vet Med Sci 2001, 63(5):547-552.
57. Knittel T, Kobold D, Piscaglia F, Saile B, Neubauer K, Mehde M, Timpl R, Ramadori G: Localization of liver myofibroblasts and hepatic stellate cells in normal and diseased rat livers: distinct roles of (myo-)fibroblast subpopulations in hepatic tissue repair. Histochem Cell Biol 1999, 112(5):387-401.
58. Zhao L, Burt AD: The diffuse stellate cell system. J Mol Histol 2007, 38(1):53-64.
59. Rubbia-Brandt L, Mentha G, Desmouliere A, Alto Costa AM, Giostra E, Molas G, Enzan H, Gabbiani G: Hepatic stellate cells reversibly express alpha-smooth muscle actin during acute hepatic ischemia. Transplant Proc 1997, 29(5):2390-2395.
60. Knežević M, Gledić D, Kukolj V, Knežević D, Jovanović M, Božić T, Aleksić-Kovačević S: Expression of α-SMA, desmin and vimentin in canine liver with fibrosis. Acta Veterinaria (Beograd) 2009, 59(4):361-370.

61. Aleksić-Kovačević S, Kukolj V, Kureljušić B, Marinković D, Knežević D, Ignjatović I, Jovanović M, Knežević M, Gledić D: Role of hepatic stellate cells (HSCs) in the development of hepatic fibrosis in cats with polycystic kidney disease (PKD). Acta Veterinaria (Beograd) 2010, 60(4):391-400.
62. Uetsuka K, Nishikawa S, Isobe K, Nakayama H: Histopathological characteristics of Kupffer cells and ito cells in the porcine and bovine liver. J Vet Med Sci 2007, 69(7):767-770.
63. Marinkovic D, Kukolj V, Aleksic-Kovacevic S, Jovanovic M, Knezevic M: The role of hepatic myofibroblasts in liver cirrhosis in fallow deer (Dama dama) naturally infected with giant liver fluke (*Fascioloides magna*). BMC Vet Res 2013, 9:45, doi: 10.1186/1746-6148-9-45.
64. Maria De Souza M, Tolentino M, Jr., Assis BC, Cristina De Oliveira Gonzalez A, Maria Correia Silva T, Andrade ZA: Pathogenesis of septal fibrosis of the liver. (An experimental study with a new model.). Pathol Res Pract 2006, 202(12):883-889.
65. Ide M, Yamate J, Kuwamura M, Kotani T, Sakuma S, Takeya M: Immunohistochemical analysis of macrophages and myofibroblasts appearing in hepatic and renal fibrosis of dogs. J Comp Pathol 2001, 124(1):60-69.
66. Mekonnen GA, Ijzer J, Nederbragt H: Tenascin-C in chronic canine hepatitis: immunohistochemical localization and correlation with necro-inflammatory activity, fibrotic stage, and expression of alpha-smooth muscle actin, cytokeratin 7, and CD3+ cells. Vet Pathol 2007, 44(6):803-813.
67. Golbar HM, Izawa T, Juniantito V, Ichikawa C, Tanaka M, Kuwamura M, Yamate J: Immunohistochemical characterization of macrophages and myofibroblasts in fibrotic liver lesions due to *Fasciola* infection in cattle. J Vet Med Sci 2013, 75(7):857-865.

MIOFIBROBLASTI U NORMALNOJ I FIBROTIČNOJ JETRI KOD RAZLIČITIH ŽIVOTINJSKIH VRSTA

KUKOLJ Vladimir

Miofibroblasti su ćelije koje imaju različito poreklo i imaju karakteristike i fibroblasta i glatko-mišićnih ćelija. Miofibroblasti potiču iz različtih izvora kao što su rezidentne mezenhimske ćelije, proces epitelno-mezenhimske tranzicije, kao i od cirkulišućih fibroblastima sličnih ćelija koje vode poreklo od matičnih ćelija ili drugih prekursora iz kostne srži. U normalnim uslovima fibroblastične ćelije pokazuju malu sposobnost da proizvode vanćelijski matriks. Nakon oštećenja tkiva, pod dejstvom citokina koji su oslobođeni iz inflamatornih i rezidentnih ćelija, one se aktiviraju i migriraju u oštećeno tkivo gde sintetišu komponente vanćelijskog matriksa. Ispitivanja citoskeletnih i površinskih ćelijskih markera pokazala su izvesni stepen heterogenosti ovih ćelija. Razlog za ovo leži u činjenici da ekspresija ovih markera u velikoj meri zavisi od vrste životinje, starosti i stepena razvoja fibroze. Bolje poznavanje molekularnih mehanizama koji su uključeni u process diferencijacije miofibroblasta tokom različitih patoloških stanja omogućava da se proces nastanka fibroze kao i njena prevencija i terapija bolje razumeju.