Professor Toshio Ito: a clairvoyant in pericyte biology

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Abstract. Ito cells are liver-specific pericytes which were first described as Fett Speicherung Zellen, the fat-storing cells encircling outside sinusoidal endothelial cells, in 1951 by the late professor Toshio Ito. His pioneering approaches for morphological characterization of the cells stimulate investigators to further examine their functional roles in liver homeostasis: a body of evidence has been accumulated in recent years showing that the cells play a crucial role in storage and delivery of vitamin A, regulation of sinusoidal tone and local blood supply, and tissue repair and fibrosis. It is now widely accepted that microvascular pericytes including Ito cells serve as a key player that controls angiogenesis. Furthermore, recent studies support a concept that Ito cells constitutes a bridging apparatus mediating bidirectional metabolic interactions between sinusoids and hepatocytes, utilizing prostanoids and/or gaseous mediators such as nitric oxide and carbon monoxide as signaling molecules. This article reviews researches on this liver-specific pericyte and its leading roles in recent development of pericyte biology.
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Key words: pericytes, microcirculation, vitamin A, carbon monoxide, soluble guanylate cyclase

Introduction

Besides hepatocytes in its parenchyma, the vertebrate liver consists of several different kinds of nonparenchymal cells, such as endothelial cells which line sinusoids, Kupffer cells or liver macrophages, pit cells or liver-associated natural killer cells, and the perisinusoidal stellate cells. The stellate cells occuring in the space of Disse were first described by von Kupffer in 1898.[1] He described that the cells were characterized by cytoplasmic inclusion bodies stained with gold chloride method, and incorrectly suggested that the gold chloride-reactive cells were phagocytic cells. According to his description, the inclusion bodies were fragments of erythrocytes taken up from circulation by phagocytosis. In 1951, when the Keio Journal of Medicine started its history, Professor Toshio Ito precisely described that the cells in the perisinusoidal space are characterized by abundant fat droplets and differ from Kupffer cells, publishing as a form of abstract[2] and as an article in the next year.[3] The cells are characterized by well-developed rough endoplasmic reticulum and lipid droplets containing vitamin A which are mainly stored in the perikaryon. Several thick cytoplasmic processes are protruded directly from the perikaryon being called the primary processes; they extend their processes on the outer surface of sinusoidal endothelial cells.[4] The cell was therefore named Ito cell after his discovery, while the same cell has also been called by a variety of different names such as pericytes, fat-storing cells, parasinusoidal cells, lipocytes and hepatic stellate cells. Since then, varied functions of this liver-specific pericytes were examined extensively through molecular biological techniques, and the results suggest an important role of Ito cells in homeostasis of hepatobiliary functions. Furthermore, his discovery of this unique cell greatly contributed to extensive advances in biology of microvascular pericytes, the definition of which was not fully established in his era. This article reviews physiologic and pathophysiologic roles of Ito cell in

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Fig. 1 Professor Toshio Ito; A portrait (cited from *Cogito* 58 MITA-HYORON No. 926 June 1991 with permission).

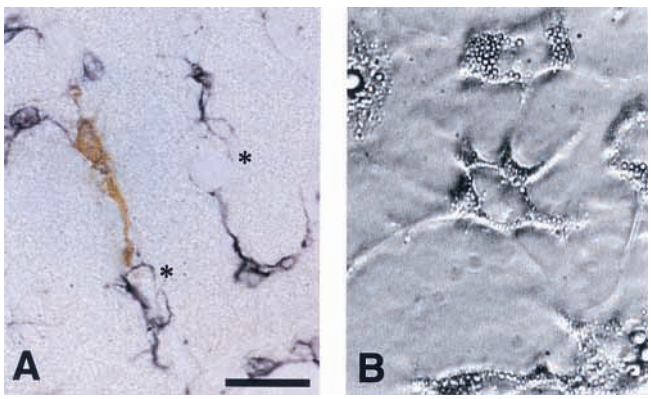

Fig. 2 A representative picture of Ito cells. Panel A: Microtopographic relation between Kupffer cells and Ito cells in the rat liver. The cells stained brown exhibit immunoreactivities to the MoAb KiM2R, a marker of macrophages, while those in purple were stained with anti-desmin MoAb as a marker of Ito cells. Note that processes of Ito cells encircle sinusoidal vessels (askterisk). Bar = 20 μm. Panel B: A representative picture of primary cultured Ito cells isolated from the rat liver. Note abundant fat droplets in the cell bodies.

liver homeostasis and highlights their significance in the pericyte biology.

How Did Professor Ito Meet His Cell?

Professor Ito graduated from Keio University School of Medicine in 1930 and started his research carrier in Department of Anatomy under supervision by Professor Okajima (Fig. 1). He moved to Tokyo Women's Medical College as Professor of Anatomy on April 1941, and spent most of his time in this position under World War II. In 1948, he took up professorship in Gunma University, where he met his cell; through light-microscopic observation, he found interesting cells characterized with abundant fat droplets that were obviously different from Kupffer's phagocytic cells and called them fat-storing cells. He reported this discovery in the 55th Annual Meeting of Japanese Society of Anatomy in 1950.[2] It should be noted that, upon his report in the meeting, Professor Tokuyasu Kudo Department of Anatomy in Niigata University School of Medicine asked an important question based on his research experiences of microtopography of vitamin A in the body, ''If your cells are characterized by abundant fat droplets, they should store lipophilic vitamins such as vitamin A!''. In these days, a majority of researchers including Professor Kudo believed that Kupffer cells constitute a major cellular component for storage of vitamin A in the liver. In 1952, these findings were published in Okajima Folia Anatomica Japonica which was extensively cited by foreign researchers over the world.[3] In 1959, his group carried out electron-microscopic observation of fat-storing cells and demonstrated that the cells are wrapped with collagen fibers and located in the outer surface of sinusoidal endothelium. Processes to establish structural characterization of the fat-storing cells were described in detail in his review article.[4] Symposia for sinusoidal cells were organized in the Netherlands in 1977 and 1982 by Professors E. Wisse and Knook DL. In these meetings, fat-storing cells were fully recognized as the third sinusoidal cells besides Kupffer cells and sinusoidal endothelial cells, as Professor Wisse cited Professor Ito and his group's investigations on the cells. Approximately 30 years had passed for such an international recognition of the work since he first described his cell.

How Can We Assess Cell Function of Pericytes *in vitro*?

Despite morphometrical one, functional characterization and its link to molecular events required another 20 years until now and still remain to be completed. From a viewpoint of vascular biology, Ito cells are considered to be microvascular pericytes which occur specifically in the liver. Pericytes, also known as Rouget cells or mural cells, are associated abluminally with true capillaries and post-capillary venules among all organ microvascular systems.[5] Differences in pericyte morphology and distribution among vascular beds suggest tissue-specific functions. Ito cells are characterized by their dendrititic structures with cytoplasm filled with vitamin A-containing fat-storing droplets (Fig. 2). Based on their complement of muscle cytoskeletal proteins, pericytes have been proposed to play a

role in the regulation of microvascular blood flow.[6,7] *In vitro* studies demonstrating the contractile ability of pericytes support this concept.[6] Pericytes have also been suggested to differentiate multipotentially into varied types of cells such as adipocytes, osteoblasts and myofibroblasts. The mechanisms involved in vessel formation have yet to be elucidated but observations indicate that the primordial endothelium can recruit undifferentiated mesenchymal cells and direct their differentiation into pericytes in microvessels, and smooth muscle cells in large vessels. Communication between endothelial cells and pericytes may take many forms. Soluble factors such as platelet-derived growth factor and transforming growth factors-β are likely to be involved.[8] In addition, physical contact mediated by cell adhesion molecules,[8] integrins and gap junctions[9] appear to contribute to the control of vascular growth and function. Ito cells share such properties of pericytes. Development of culture methods has allowed some functions of pericytes to be directly examined. When primarily cultured, Ito cells build up intercellular communications by forming gap junctions that greatly contribute to electrophysiologic coupling between the adjacent cells (Fig. 3). Co-culture of pericytes with endothelial cells leads to the activation of transforming growth factor-β, which in turn influences the growth and differentiation of the vascular cells. Furthermore, contractility of pericytes has been examined directly through ''wrinkling assay'' using culture dishes coated with silicon membrane;[6] upon exposure to vasoconstrictive mediators such as angiotensins or endothelins, the ability of cultured pericytes to produce contractile force is able to be judged by formation of silicon wrinkles around the cells. Another methodology to examine the cellular contractile force is collagen lattices contraction assay.[10,11] These two methods have widely been used to study function of Ito cells and thus remind investigators that such contractile properties of the cells could play roles in regulation of local blood flow and tissue contraction upon wound healing during fibrosis.

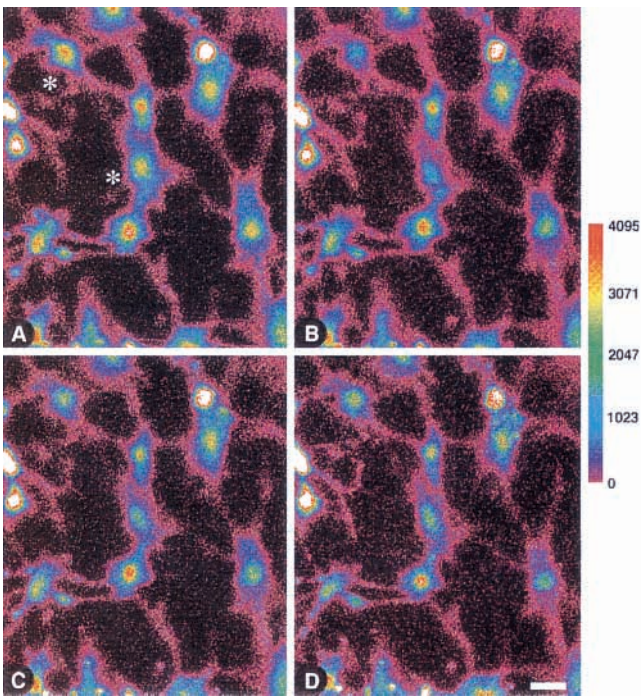

Fig. 3 Representative pictures of fluorescence recovery after photobleaching in a single Ito cell in culture preloaded with carboxyfluorescein, indicating the presence of gap junctional communications between the adjacent cells. Asterisks indicate cells undergoing laser photobleaching. Serial microfluorographs were recorded before (A), and 0 min (B), 2 min (C), and 6 min (D) after completing the laser photobleaching. Sequence of the color bar 8right) denotes the fluorescence intensities. Bar = 20 μm. Cited from Am J Physiol (reference 8) with a permission.

Ito Cells As A Center for Vitamin A Metabolism

The liver is known to store approximately 80% of vitamin A in the body. Ito cells constitute a major cellular component executing its storage in the liver. The cells distributed almost homogeneously throughout different zones of the liver lobule, however, Ito cells in pericentral regions tend to store far less vitamin A than those in periportal regions.[12,13] On the other hand, Ito cells are currently regarded as the principal cell type responsible for matrix accumulation during repair reactions of the damaged liver that are altered as a function of vitamin A contents in the cells.[13] A variety of reagents causing liver damages or fibrosis such as carbon tetrachloride and PCB, one of the major environmental hormones, and chronic ethanol administration have been known to reduce the vitamin A contents in the cells.[13] Structure and function of the cells have thus been revealed with advances of researches on pathogenesis of liver fibrosis. A basic feature of the response of Ito cells to hepatic tissue injury is called ''activation''. Such activation processes involve cell proliferation, transformation from star-shaped, vitamin A-rich cells to vitamin A-deficient cells with a myofibroblast-like appearance displaying increased contractile properties. These results suggest that a shortage of vitamin A or metabolites of stored retinoids could cause transformation of the cell types. Furthermore, activation is characterized by differential gene expression of connective tissue components, matrix-degrading enzymes, and their inhibitors, resulting in matrix accumulation colocalized with activated Ito cells.[14] Interestingly, this *in vivo* activation process strongly resembles the morphological and functional changes observed in Ito cells during primary culture, and therefore the cells *in vitro* are commonly used as a model to

study the role of them during hepatic tissue repair. Under quiescent conditions, Ito cells are unlikely to express voltage-operated Ca^{2+} or Na^{+} channels which commonly play a role in cell contraction in other cell types such as myocytes and vascular smooth muscle cells. With a reduction of vitamin A storage, the cells in culture increase their expression of α-smooth muscle cell actin and voltage-dependent calcium channels, indicating a contractile phenotype.[9] Details of metabolism and mobilization of vitamin A in and around Ito cells were described in previous review articles.[11]

Molecular Markers Altering in Parallel with Ito Cell Activation

Several extracellular stimuli, including, e.g. inflammatory cytokines, growth factors, vasoactive peptides, and extracellular matrix components, as well as a number of intracellular signaling pathways, are involved in the activation process of Ito cells. However, the overall picture is far from complete, and the molecular mechanisms regulating Ito cell activation, particularly at the transcriptional level, are still under investigation. To address this question recent studies used differential mRNA display technologies and proteomics analyses in the cells at different stages of *in vitro* activation to identify key regulators involved in this activation process.[14–16]

With the former technique the transcription factor Ets-1 was detected through its down-regulation during Ito cell activation. Ets-1 has been known as an important role in cell proliferation, differentiation, development, transformation, angiogenesis, and apoptosis. The Ets DNA-binding motif, GGA(A/T), has been found in numerous genes, including transcription factors, receptor-type kinases, and proteases. Among the proteases, stromelysins, collagenase, and urokinase plasminogen activator are typical Ets-1-responsive genes. Interestingly, all of the latter proteins are expressed by Ito cells in the early phase of primary culture. Apart from direct DNA binding as monomers, Ets-1 cooperates with various transcriptional activators such as the AP-1 family in regulating gene activity and has been shown to activate gene transcription through a Ras-stimulated signal-transducing pathway that includes MAP kinases.[15]

Ito Cells: A Metabolic Bridge between Sinusoids and Parenchyma?

Liver utilizes a variety of biologically active signaling molecules to execute intercellular communications between different cell types. Recent studies support a concept that Ito cells serve a metabolic bridge that constitutes bidirectional signaling pathways between sinusoids and parenchymal cells. A typical example for the role of Ito cells in alterations of sinusoidal function through hepatocyte-mediated mechanisms is a reception of carbon monoxide (CO) by soluble guanylate cyclase in Ito cells that contributes to maintenance of sinusoidal patency.[17] CO is a by-product of the reaction of heme oxygenase (HO) that oxidatively catalyzes protoheme-IX into biliverdin-IX α.[18] Liver constitutes a major organ responsible for detoxification of the hemoglobin-derived heme and biliary excretion of bilirubin-IX, a product generated from biliverdin-IX through biliverdin reductase.[19] We examined intrahepatic distribution of two major HO isozymes immunohistochemically, with the finding that the two isozymes have distinct topographic patterns; HO-1, the inducible form, is expressed prominently in Kupffer cells, while the constitutive HO-2 is abundant in hepatocytes.[16] We have shown that CO derived from HO-2 is necessary to keep sinusoids in a relaxing state through mechanisms involving soluble guanylate cyclase in Ito cells. In other words, Ito cells utilize soluble guanylate cyclase to sense CO for sinusoidal relaxation.[7,19] Considering the microanatomical orientation of the liver cells in and around sinusoids, HO-2 in parenchyma stands in the reasonable position for the gas reception by Ito cells where CO released from hepatocytes can directly access to the cells and thereby modulate their contractility without being captured by hemoglobin in circulation.[17] When exposed to disease conditions such as endotoxemia and advanced cirrhosis, liver could upregulate HO-1 in Kupffer cells and hepatocytes as a result of cytokine responses.[20,21] In experimental models of endotoxemia, such an induction of HO-1 expands the ability of liver to degrade heme and to generate CO. Under these circumstances, CO turned out to contribute to maintenance of blood perfusion as well as bile excretion.[21]

Besides CO-mediated vasorelaxation, a variety of vasoactive mediators for controlling sinusoidal tone have been studied extensively. Such substances involve endothelins, thrombin and ATP as ''contractile'' mediators for sinusoids, and prostaglandin E_2, nitric oxide (NO) as ''relaxing'' mediators. Among these substances, endothelins, NO and prostaglandin (PG) E_2 constitute those which transfer information at the sinusoidal side towards hepatocytes.[22] In unstimulated livers, endothelins and NO are generated in sinusoidal endothelial cells. Ito cells are thought to express both ET_A and ET_B receptors and contribute to cell contraction upon the ligand binding.[23,24] Since ET_B receptor is also expressed in sinusoidal endothelium and elicits NO generation, this pathway could contribute to Ito cell relaxation. Although most of previous observations suggest that blockade of endothelin-mediated signaling pathways attenuates liver injury induced by varied dis-

ease conditions, of interest is a recent observation suggesting that blockade of ET_B receptor could suppress endothelial NO generation and thereby aggravate reoxygenation injury of the liver, raising a possible protective role of this pathway in maintenance of the physiological functions.[25]

PGE_2 has recently been shown to be released from Kupffer cells in response to administration of ethanol or endotoxin and thereby stimulate oxygen uptake and induce glycogenolysis in hepatocytes.[26] At the same time, Ito cells are known to exhibit relaxation in response to PGE_2 presumably through the cAMP-dependent mechanism. Many of such biological effects of PGE_2 are mediated through its interactions with specific receptors including EP2 and EP4 receptors, which function *via* Gs-proteins to increase cAMP.[27] Although PGE_2-mediated physiologic link between glycogenolysis and sinusoidal relaxation is largely unknown, the cAMP-mediated relaxation of Ito cells could help sinusoids to guarantee ample blood supply for local oxygen demand and to deliver glucose into circulation. Such roles of Ito cells as a metabolic bridge for cooperative and integrative interactions between blood supply and hepatocellular metabolism should deserve further studies to understand pathophysiologic mechanisms for varied disease conditions such as decompensatory cirrhosis or primary non-function liver in transplantation.

Future Perspectives of Ito Cell Biology

The previous dogmatic aspect that Ito cells are simply desmin-positive cells with vitamin A-containing droplets is changing. Recent studies provided evidence for the presence of a variety of molecular markers including RhoN, glial fibrillary acidic protein, nestin, and neurotrophin receptors in a subpopulation of Ito cells.[14] These findings raised a fundamental question as to whether all the mesenchymal cells derive from the same embryonic source in the liver. Even more important in the related field is a concept that cellular plasticity may not be confined within the same mesenchymal lineage but rather trans-differentiation from one cell lineage to another could be possible even in adult tissues. Such a paradigm shift on pluripotentiality of Ito cells could lead to development of new therapeutic approaches on the cells to control liver fibrosis through cell reconstitution as well as to establishment of tissue engineering approaches for liver regeneration.

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