CELL STRUCTURE AND FUNCTION 25: 329–336 (2000)

REVIEW

Adhesion between Cells and Extracellular Matrix with Special Reference to Hepatic Stellate Cell Adhesion to Three-dimensional Collagen Fibers

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ABSTRACT. **Hepatic stellate cells are located in the perisinusoidal space (space of Disse), and extend their dendritic, thin membranous processes and fine fibrillar processes into this space. The stellate cells coexist with a three-dimensional extracellular matrix (ECM) in the perisinusoidal space. In turn the three-dimensional structure of the ECM regulates the proliferation, morphology, and functions of the stellate cell. In this review, the morphology of sites of adhesion between hepatic stellate cells and extracellular matrix is described. Hepatic stellate cells cultured in polystyrene dishes spread well, whereas the cells cultured on or in type I collagen gel become slender and elongate their long cellular processes which adhere directly to the collagen fibers. Cells in type I collagen gel form a large number of adhesive structures, each adhesive area forming a face but not a point. Adhesion molecules, integrins, for the ECM are localized on the cell surface. Elongation of the cellular processes occurs via integrin-binding to type I collagen fibers. The signal transduction mechanism, including protein and phosphatidylinositol phosphorylation, is critical to induce and sustain the cellular processes. Information on the three-dimensional structures of ECM is transmitted via three-dimensional adhesive structures containing the integrins.**

Key words: hepatic stellate cells/extracellular matrix/cell-extracellular matrix adhesion/type I collagen/three-dimensional structure

The extracellular matrix (ECM) is known to regulate a diversity of biological activities, including cell proliferation, differentiation, migration, polarity, tumorigenesis and cancer metastasis. The cells adhere to ECM by specialized cellular devices such as focal contacts, podosomes, point contacts, and hemidesmosomes (Senoo and Hata, 1994b).

The ECM is composed of an insoluble complex of collagens, adhesive glycoproteins (e.g., fibronectin, laminin), proteoglycans (PGs) (e.g., heparan sulfate PGs, chondroitin sulfate PGs) and elastin. These ECM components construct an insoluble supramolecular complex (reviewed by Hata, 1996), the molecules of which contain positional information relayed back as signals to the cells that produce them or to neighboring cells, as well as regulate their growth and metabolism (reviewed by Hata, 1996).

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Abbreviations: ECM, extracellular matrix; PGs, proteoglycans: SEM, scanning electron microscopy; TEM, transmission electron microscopy; EHS, Engelbreth-Holm-Swarm; DGEA, Asp-Gly-Glu-Ala; FAK, focal adhesion kinase; PI3-kinase, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase.

Integrins are the major family of cell surface receptors that mediate attachment to the ECM (Hynes, 1992; Clark and Brugge, 1995; Giancotti and Ruoslahti, 1999). Formation of focal contacts is regulated by intracellular signals which promote integrin clustering and cytoskeletal associations. The relationship between the cells and ECM is one of the most important aspects with regard to the nature of the cells.

Hepatic stellate cells (vitamin A-storing cells, lipocytes, fat-storing cells) lie in the perisinusoidal space (space of Disse) (Wake, 1980, 1995, 1997) and coexist with the three-dimensional ECM components within the space. The types of ECM components regulate the behavior of the stellate cell (Senoo *et al*., 1991; Senoo and Hata, 1994a, 1994b, 1995). The three-dimensional structure of substratum also reversibly regulates the morphology, proliferation rate and function of cultured hepatic stellate cells (Senoo *et al*., 1996, 1997, 1998; Kojima *et al*., 1997; Imai *et al*., 1997). Adhesion between the stellate cells and ECM is essential in the regulation of the cells by ECM.

The stellate cells extend their thin membranous processes, dendritic processes, and fine fibrillar processes into the

perisinusoidal space and coexist with the three-dimensional ECM components within the space (Wake, 1980). The long three-dimensional processes of the stellate cells and three-dimensional ECM form a complicated structure. Hence, it is difficult to analyze relationship between hepatic stellate cells and ECM *in vivo* (Imai and Senoo, 2000).

In this review, the morphology of adhesion between cultured stellate cells and three-dimensional substratum is discussed with special reference to cell adhesion to three-dimensional collagen fibers and adhesive molecules, namely, integrins.

Hepatic stellate cell

Hepatic stellate cells are the only one mesenchymal fixed cell type located perisinusoidally (Wake, 1997). The stellate cells have two characteristic morphological features.

Lipid droplets in the cytoplasm are the most characteristic ultrastructural feature of the stellate cells (Wake, 1971, 1980; Blomhoff *et al*., 1990; Imai *et al*., 2000). These droplets contain vitamin A (Wake, 1971, 1980; Blomhoff *et al.*, 1990; Senoo and Hata, 1993; Senoo *et al*., 1997). In mammals, hepatic stellate cells store about 50 to 80% of the total body vitamin A as retinyl esters in the lipid droplets in the cytoplasm (Blomhoff, 1990). In physiological condition, these cells play pivotal roles in the regulation of retinoid homeostasis (Blomhoff *et al*., 1990; Senoo *et al*., 1990, 1993, 1997).

The three-dimensional cell form of the hepatic stellate cells can be visualized by means of the Golgi method (Wake, 1993). Two different types of cellular processes can be distinguished. Hepatic stellate cells bear long branching cytoplasmic processes with numerous thorn-like microprojections extending from them. Scanning electron microscopic examination reveals that the spines extend from the lateral edge of the subendothelial cytoplasmic processes and pass direct obliquely through the space of Disse away from the abluminal surface of the endothelial cells to make contact with the plasma membrane of the parenchymal cells (Wake, 1995). Thus the stellate cells adhere to the endothelial cells on one hand and the other hand make contact with the parenchymal cells (reviewed by Wake, 1980).

ECM in the perisinusoidal space

The three-dimensional structure of the sinusoidal wall and the perisinusoidal space were examined by computer assisted reconstruction of serial sections of Golgi stained preparations, and by transmission and scanning electron microscopy. The sinusoidal wall consists of three cell-layers: a Kupffer cells and liver-associated lymphocytes layer, an endothelial celllayer and the perisinusoidal stellate cell layer (Wake, 1995). Basement membrane-like structures interruptedly lie between the endothelial cell layer and the stellate cell layer (Wake, 1997). Small narrow strands of material resembling a basement membrane can be discerned beneath the endothelial lining or between the lining and the underlying subendothelial projections of the stellate cells (Wake, 1980). The stellate cells appear to adhere to the sinusoid wall by the basement membrane. Collagen fibrils pass through the tunnels formed by the processes of the stellate cells and the parenchymal cells (Wake, 1995). Thus the stellate cells adhere to the basement membrane-like matrix on one hand and the other hand are in contact with the collagen fibrils.

Normal perisinusoidal space contains type I, III, IV, V, and VI collagens, fibronectin, laminin, and proteoglycans (Martinez-Hernandez, 1984; Geerts *et al*., 1986, 1990, 1994; Rojkind and Greenwel, 1994; Martinez-Hernandez and Amenta, 1993a, 1995). A matrix gradient in the space of Disse has been revealed by detailed and extensive ultrastructural and immunochemical studies (Reid *et al*., 1992; Sigal *et al*., 1992).

Fibronectin is present mainly in amorphous material in close contact with the microvilli of the parenchymal cells (Geerts *et al*., 1986; Martinez-Hernandez and Amenta, 1993b, 1995). Heparan sulfate proteoglycan is present in diffuse material associated with the surface of the parenchymal and endothelial cells (Geerts *et al*., 1986).

Immunofluorescence for type I collagen has been found to correspond with some of the coarse reticular fibers, while that for type III collagen has been found to correspond with most, but not all, reticular fibers of the liver in the snow monkey (Adachi *et al*., 1991). By immunoelectron microscopy, reaction products with anti-type III collagen antibody and anti-type V collagen antibody have been demonstrated on cross-striated collagen fibrils, about 45 nm in diameter, in the space of Disse in monkey liver. From these observations, it has been concluded that: (1) the fine reticular fibers are mainly composed of type III and V collagens, and (2) the collagen fibers and coarse reticular fibers in the periphery of liver lobules are composed of type I, III and V collagens. Simultaneous localization of type I collagen and type III collagen in the same cross-striated collagen fibril has also been revealed in the space of Disse of normal rat (Geerts *et al*., 1986, 1990). In either case, each ECM component localizes three-dimensionally in a restricted region in the normal liver lobule.

Under pathological conditions, such as liver fibrosis, activated hepatic stellate cells lose their lipid droplets containing vitamin A, proliferate rapidly, and synthesize and secrete a large amount of ECM components such as type I, III, IV collagens, fibronectin, laminin, heparan sulfate, and dermatan sulfate (Clement *et al*., 1986; Gressner and Bachem, 1990, 1995; Ramadori, 1991; Geerts *et al*., 1994; Greenwel *et al*., 1994; Gressner *et al*., 1994; Martinez-Hernandez *et al*., 1995). The constitution of ECM components in the liver lobules and in the perisinusoidal space is altered according to pathological conditions (Gressner and Bachem, 1995). As the liver becomes fibrotic, the total content of collagens

and noncollagenous components increases 3- to 5-fold (Friedman, 2000). Therefore the three-dimensional ECM in the space of Disse is not static but dynamic.

Behaviors of the stellate cells are regulated by three-dimensional ECM

Behaviors of the stellate cells are regulated by the type of ECM components (Senoo *et al*., 1991; Senoo and Hata, 1994a, 1994b, 1995). Cultured hepatic stellate cells proliferate better and synthesize more collagen in type I collagen-coated culture dishes than in polystyrene dishes. On a basement membrane gel prepared from Engelbreth-Holm-Swarm (EHS) murine tumor, the cultured stellate cells form a mesh-like structure, proliferate slowly, and synthesize a small amount of collagen. Cultured rat and human stellate cells exhibit three different types of morphology according to the substratum used for culture (Sato and Senoo, 1998). When the stellate cells are cultured on the polystyrene surface or in type I, III, or IV collagen-coated culture dishes, the cells have a flattened shape with well-developed stress fibers (Tomasek *et al*., 1982). When the cells are cultured on type I or type III collagen gel, the cells elongate their cytoplasmic processes. After long term (over a week) culture in type I collagen gel, the cultured stellate cells display three-dimensionally developed processes with numerous secondary and tertiary fine branches, similar to those of glial or neuronal cells. By contrast, when stellate cells are cultured in or on Matrigel, the cells maintain a round shape and show no signs of either cell spreading or elongation of processes (Senoo, and Hata, 1994a, 1994b; Sato and Senoo, 1998).

Recently, we found that the three-dimensional structure of substratum reversibly regulates morphology, proliferation rate and function of cultured hepatic stellate cells (Senoo *et al*., 1996, 1997, 1998; Kojima *et al*., 1997; Imai *et al*., 1997). The molecular mechanisms in the regulation of stellate cells by three-dimensional structure of the ECM imply that cell-surface integrin binds to the matrix components followed by signal transduction processes and cytoskeleton assembly (reviewed by Senoo *et al*., 1998). However, the morphology of the site of adhesion between ECM components and hepatic stellate cells has not been thoroughly examined.

Very recently, we investigated the morphology of adhesion between ECM and cultured stellate cells by both scanning (SEM) and transmission-electron microscopy (TEM) (Imai and Senoo, 1998). We will discuss here the morphology of sites of adhesion between cultured stellate cells and three-dimensional substratum with special reference to collagen type I and adhesive molecules, namely, integrins.

Morphology of adhesion between the stellate cells and ECM

When the stellate cells isolated from the liver of an adult male Wistar rat and cultured in non-coated polystyrene culture dishes, the cells spread well and became flat (Fig. 1a and b) (Imai and Senoo, 1998). The size of cells was 50–100 μm (diameter). Only a few lipid droplets were retained within the cytoplasm (arrowhead in Fig. 1a). SEM examination showed some lamellipodia in the peripheral portion of the cells (arrows in Fig. 1b).

When stellate cells were inoculated on rigid type I collagen gel, the cells extended and became long polygonal (Fig. 1c). The cells retained small lipid droplets (arrowhead in Fig. 1c) within the cytoplasm and resembled the star-shaped stellate cells as found *in vivo*. Cells cultured on type I collagen gel extended their primary (arrows in Fig. 1d) and secondary long cellular processes (arrowheads in Fig. 1d) from the cell bodies onto the gel (Fig. 1d). These processes became entangled and made contact with the fine type I collagen fibers of the gel.

When the stellate cells were inoculated into type I collagen gel and cultured for 2 days, the cells were rod- or long spindle-like (arrow in Fig. 1e) and 100–300 μm in length. By SEM examination at a lower magnification, the cell bodies (arrow in Fig. 1f) and cellular processes of the stellate cells cultured in the gel are seen to be entangled with the three-dimensional collagen fibers of the gel. The collagen fibers attached not only to the lower side but also the upper side of both cell bodies and the cell processes. These data indicate that the stellate cells cultured on or in type I collagen gel extend long cellular processes from their cell bodies whereas the cells cultured in non-coated polystyrene culture dishes spread well and become flat. The stellate cells in the type I collagen gel become entangled three-dimensionally with the collagen fibers and make contact with the fibers. These data strongly suggest that hepatic stellate cells adhere directly to three-dimensional ECM.

The three-dimensional collagen fibers (arrowheads in Fig. 2a) of type I collagen gel make contact with cell bodies, cell processes, and a number of microprojections (arrows in Fig. 2a) of the stellate cells cultured in the collagen gel. These contacts are clearly evident by SEM examination of the cells at higher magnification (Fig. 2a).

The morphology of the site of adhesion between networks of collagen fibers and the stellate cells in type I collagen gel is revealed more precisely by TEM observation (Fig. 2b and c). The collagen fibers (arrowheads in Fig. 2b and c) adhere directly to the surfaces of the cell bodies (Fig. 2c), cellular processes, and their thin microprojections. The area of these adhesions between the type I collagen fibrils and the stellate cells formed a face but not a point at the electron microscopic level (Fig. 2b and c). The adhesive face measured 50 to 1,000 nm in length in thin TEM sections. Accumulations of actin-like thin filaments are ob-

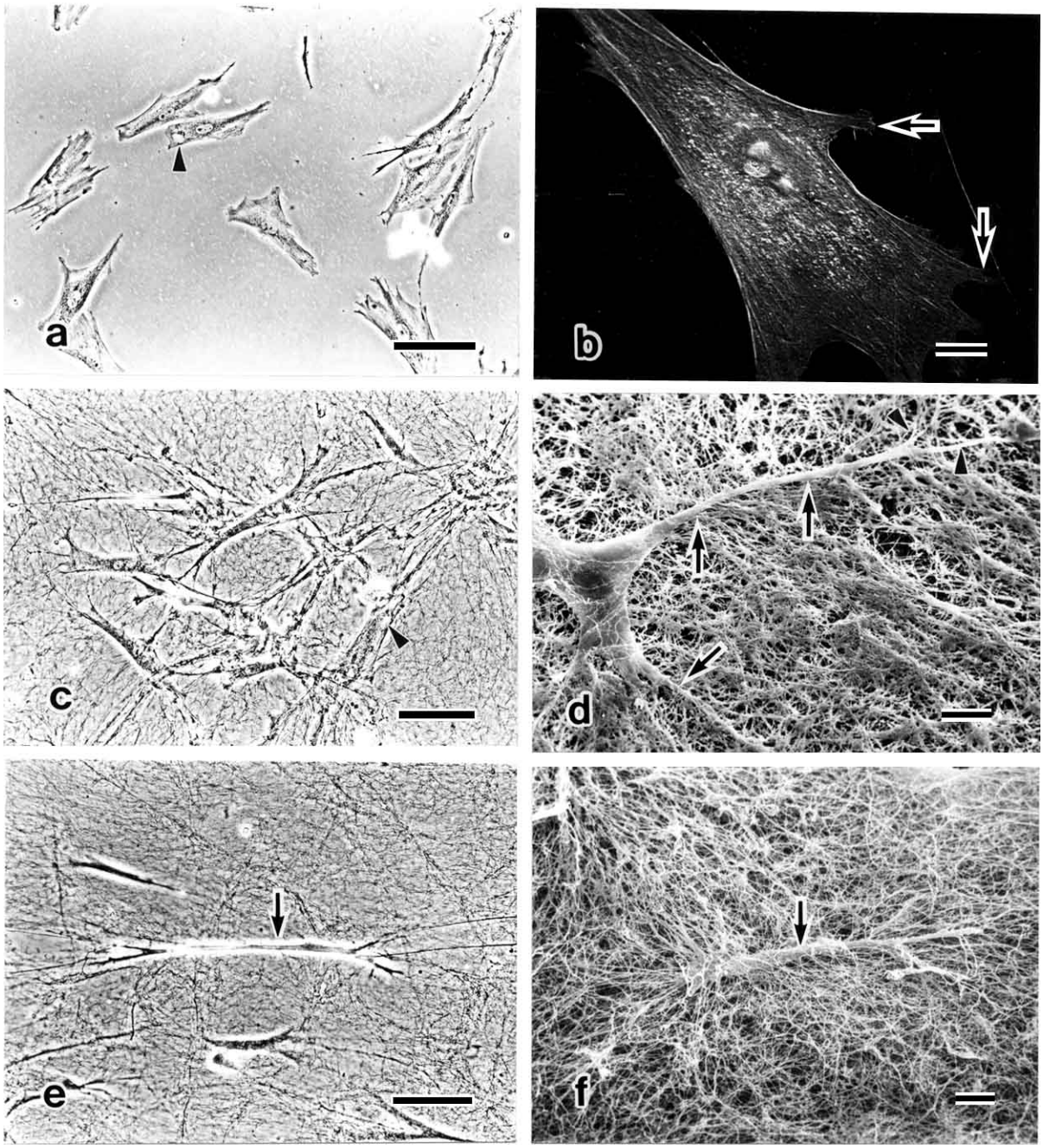

Fig. 1. Morphology of rat hepatic stellate cells cultured in a non-coated polystyrene culture dish (**a** and **b**), on (**c** and **d**), or in (**e** and **f**) type I collagen gel for 2 days. Panels **a**, **c** and **e** show phase-contrast photomicrographs of the cultured stellate cells. Panels **b**, **d**, and **f** show scanning electron micrographs of the cultured stellate cells. Photomicrographs of the stellate cells were taken as described (Imai and Senoo, 1998). **a**: The stellate cells cultured in a non-coated polystyrene culture dish spread well on a base of the tissue culture dish. An **arrowhead** indicates a lipid droplet within the cytoplasm of the cell. ×240. **b**: A stellate cell cultured in a non-coated polystyrene culture dish spread well and formed some lamellipodia (**arrows**) in the peripheral portion of the cell. ×760. **c**: The stellate cells cultured on type I collagen gel are long polygonal. The cells protruded cytoplasmic processes from the cell bodies. A small lipid droplet (**arrowhead**) is observed within the cytoplasm of the cell. ×240 **d**: A stellate cell cultured in type I collagen gel extends long primary cellular processes (**arrows**) and secondary processes (**arrowheads**). These processes contact with fine type I collagen fibers. ×760. **e**: The stellate cells (**arrow**) cultured in type I collagen gel show rod- or long spindle-like shape and extend their long cellular processes into type I collagen gel. ×240 **f**: A stellate cell (**arrow**) cultured in type I collagen gel is covered with a network of collagen fibers. ×380. Bars indicate 50 μm (a,c,e), and 10 μm (b,d,f).

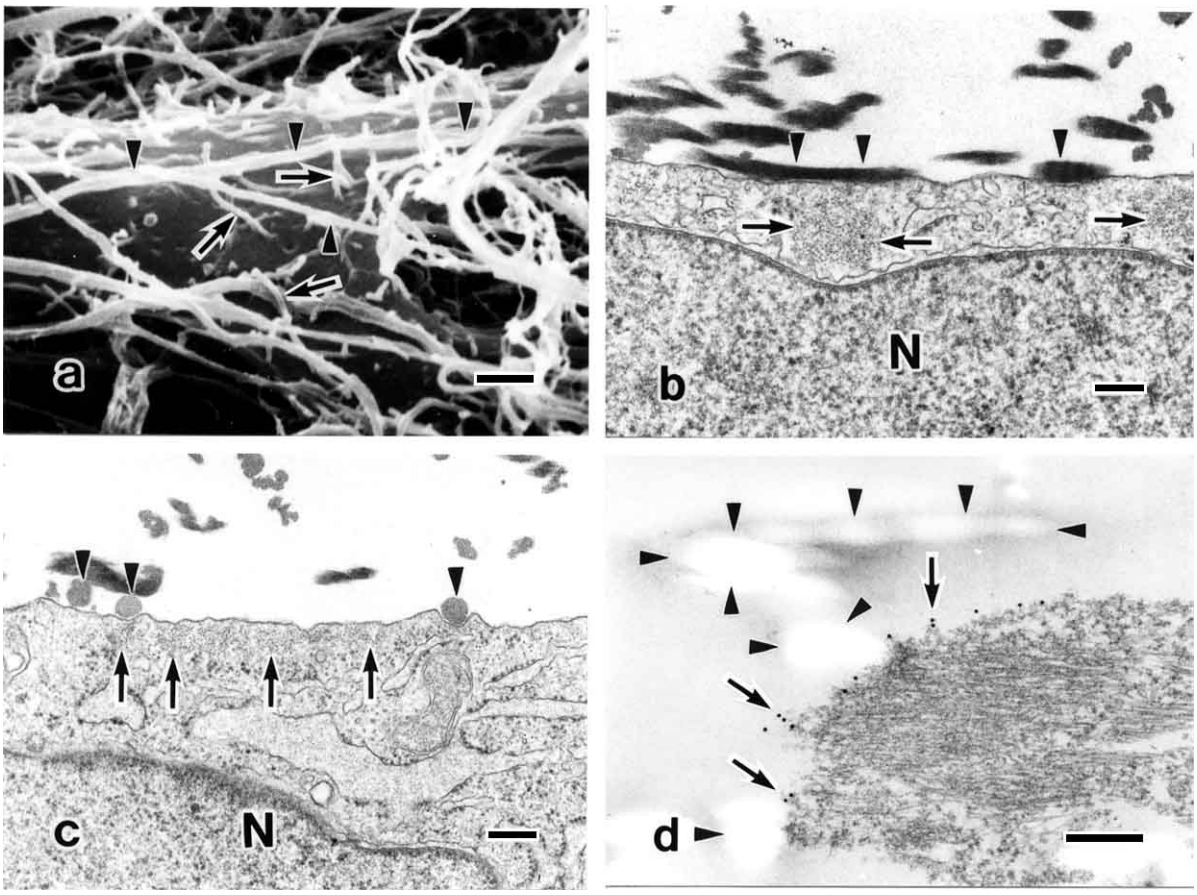

Fig. 2. Morphology of adhesion of cultured rat stellate cells to collagen fibers and localization of integrin molecules of cultured human stellate cells in type I collagen gel. Photomicrographs of the stellate cells were taken as described (Imai and Senoo, 1998). **a**: Scanning electron micrograph of the same cell cultured in type I collagen gel as shown in **Fig. 1f** at higher magnification. Collagen fibers (**arrowheads**) run along the surface of the cell. Microprojections (**arrows**) protrude from the surface of the cell. ×7,600. **b** and **c**: Transmission electron micrographs of rat stellate cells cultured in type I collagen gel. Type I collagen fibers (**arrowheads**) run along the surface of the cells and adhere directly to the surface. The area of the adhesion formed a face but not a point. These adhesive faces measured 50–1,000 nm in length in thin TEM sections. Accumulations of thin filaments (**arrows**) are observed beneath the cell surface. Capital letter **N** indicates the nuclei of the stellate cells. ×28,500. **d**: Immuno-electron micrograph for integrin α2 in a cellular process of human stellate cells cultured in type I collagen gel. The cells were immunostained with a mouse monoclonal anti-human integrin α2 antibody and a goat anti-mouse IgG conjugated 10 nm colloidal gold. Electron lucent areas represent type I collagen fibers (**arrowheads**). Adhered collagen fibers and immunogold particles (**arrows**) co-localized at the process. ×4,300. Bars indicate 1 μm (a), and 200 nm (b,c,d).

served beneath the surfaces of the cell bodies (arrows in Fig. 2b and c), although the cells do not show any cytoplasmic electron-dense plaques as seen in focal adhesion.

In normal human skin fibroblasts cultured with type I collagen polymers, electron-dense areas beneath the plasma membrane of the cells are observed at several locations along the collagen fibrils (Mercier *et al*., 1996). Measurements of these structures indicate that they are 252±36 nm long and 27±7 nm thick, which confirms that fibroblasts develop focal contacts in the presence of collagen polymers. Embryonic avian corneal fibroblasts cultured in type I collagen gel do not develop either adhesion plaque or stress fibers, although the cells cultured on non-coated cover slip develop both electron-dense plaques and distinct stress fibers (Tomasek *et al*., 1982). Thus there are some morphological differences in the adhesive structure between cells and collagen fibers, which may be due to species and cell type differences.

Adhesion molecules for ECM in the stellate cells

Integrins comprise a large family of transmembrane adhesion proteins composed of an α and a β subunit (Hynes, 1992; Giancotti, 2000). At least 24 integrin heterodimers, comprised of 9 types of β subunits and 16 types of α subunits, have been identified. Most integrins recognize several ECM proteins. Conversely, individual matrix proteins bind to several integrins; for example, at least eight integrins (α5β1, α4β1, α4β7, αvβ1, αvβ6, αvβ8, αvα3, and αIIb β3) bind to fibronectin and five integrins (α1β1, α2β1,

α3β1, α6β1, and α6β4) bind to laminin (Giancotti and Ruoslahti, 1999; Giancotti, 2000). Hepatic stellate cells express integrins α1β1, α2β1, αvβ1, α6β4, and α8β1 (Carloni *et al*., 1996; Levine *et al*., 2000). Integrin α2β1 is defined as a specific receptor for type I collagen and type IV collagen (Takada *et al*., 1988) and it can also interact with laminin and fibronectin (Elices and Hemler, 1989). The minimal active recognition sequence has been defined as a tetrapeptide of the sequence DGEA (Asp-Gly-Glu-Ala), corresponding to residues 435–438 of the type I collagen sequence (Staatz *et al*., 1991). Adhesion between human skin fibroblasts and type I collagen monomers is mediated by only two integrins, namely, α1β1 and α2β1 integrins (Mercier *et al*., 1996)

Recently, we examined the localization of integrin α2 and β1 subunits in the human stellate cells cultured in type I collagen gel by immunoelectron microscopy (Imai and Senoo, 1998). Integrin α2 and β1 subunits were detected on the surfaces of the cell bodies, cellular processes (arrows in Fig. 2d), and microprojections. Thus the information on the three-dimensional structure of the ECM is transmitted to the stellate cells by three-dimensional adhesive structures of the cells containing integrins.

Putative role of adhesion between stellate cells and ECM

Hepatic stellate cells cultured on or in type I collagen gel are induced to extend their long cellular processes, which seem to extend along the type I collagen fibrils. To study the role of integrin-binding to collagen fibrils in the extension of cellular processes, the influence of synthetic oligopeptide containing integrin-binding sequences in type I collagen and the effects of antibody against integrin α2 or β1 were examined. Synthetic oligopeptide DGEA from type I collagen molecule and anti-integrin α2 antibody inhibit the elongation of the cellular posesses (Sato *et al*., 1998). These results suggest that the elongation of the cellular processes occurs via the integrin-binding to the sequence DGEA on extracellular type I collagen fibrils (Miura, 1997; Sato and Senoo, 1998; Sato *et al*., 1998).

Ligand binding to the integrin family of cell adhesion molecules is currently thought to lead to integrin clustering and subsequent recruitment of actin filaments to the cytoplasmic domain of integrins. This recruitment is achieved via a complex of interacting cytoskeletal proteins, including talin, vinculin, α-actinin and filamin. Talin has binding sites for integral membrane proteins, cytoskeletal proteins and the protein tyrosine kinase called focal adhesion kinase (FAK). Thus, talin is likely to play a key role in coupling membrane proteins to cytoskeletal structures and signaling pathways (Critchley, 2000).

Integrins transduce signals by associating with adapter proteins that connect the integrin to the cytoskeleton and cytoplasmic kinases. Integrin signaling and assembly of the cytoskeleton are intimately linked. As integrins bind to ECM, they become clustered in the plane of the cell membrane and associate with a cytoskeletal and signaling complex that promotes the assembly of actin filaments. The reorganization of actin filaments into larger stress fibers in turn causes more integrin clustering. Integrins activate various protein tyrosine kinases including FAK, Src-family kinases, and serine-threonine kinase. FAK also combines with, and may activate, phosphatidylinositol 3-kinase (PI 3-kinase). These interactions link FAK to signaling pathways that modify the cytoskeleton and activate mitogen-activated protein kinase (MAPK) cascades (Clark and Brugge, 1995; Giancotti and Ruoslahti, 1999).

Protein tyrosine phosphorylation is enhanced in stellate cells cultured on type I collagen gel, while protein tyrosine kinase inhibitors or Pl3-kinase inhibitors retract the extended cellular processes and also inhibit the induction of cellular processes (Kojima *et al*., 1997; Miura *et al*., 1997; Sato and Senoo, 1998). Therefore, the signal transduction mechanism including protein and phosphatidylinositol phosphorylation seems to be critical to induce and sustain the cellular process architecture.

Dual immunofluorescence staining of microtubules and F-actin in stellate cells cultured on type I collagen gel has revealed that the core of elongated cellular processes of the cells consists of microtubules, while the periphery contains F-actin (Miura *et al*., 1997; Sato *et al*., 1998).

Well-developed cellular processes of cultured stellate cells are retracted in the presence of colchicine. Since cytochalasin B also destroys the cellular process architecture, the peripheral F-actin organization is necessary to sustain the microtubule core (Miura, 1997; Sato and Senoo, 1998; Sato *et al*., 1998). These results indicate that the processes are induced by cell surface integrin binding to interstitial collagen fibrils, followed by signaling processes including protein and/or phosphatidylinositol phosphorylation, and finally F-actin and microtubule organization.

In hepatic stellate cells cultured in or on type I collagen gel, time-lapse video-microscopy has demonstrated that the long processes appear to contact with and extend along collagen fibrils. The cellular processes also extended in or on type III collagen gel, but not in type IV collagen-coated dish or on Matrigel containing the basal membrane components (Miura *et al*., 1997; Sato and Senoo, 1998). Therefore, the morphological changes in stellate cells occurred by the recognition of fibrillar type I or type III collagen, but not type IV collagen or other Matrigel components.

The normal perisinusoidal space contains mainly type I and III collagen fibers (Clement *et al*., 1986) and each ECM component localizes three-dimensionally at a restricted region in the normal liver lobule (Reid *et al*., 1992; Sigal *et al*., 1992; Martinez-Hernandez and Amenta, 1995). It is therefore concluded that hepatic stellate cells in the space of Disse make multiple three-dimensional adhesive structures to three-dimensional ECM components and receive the

positional information from the ECM through the adhesive structures.

Conclusion

Cultured hepatic stellate cells adhere directly to three-dimensional fibers of type I collagen gel. Cells on or in type I collagen gel become slender and extend long cellular processes onto or into the gel, which become entangled three-dimensionally with type I collagen fibers. Cells inoculated in type I collagen gel form a large number of three-dimensional adhesive structures. Each adhesive area forms a face but not a point. Adhesion molecules specific for the ECM, namely integrin $\alpha 2$ and $\beta 1$ subunits are localized on the surface of cell bodies, cellular processes, and microprojections of the hepatic stellate cells.

Stellate cells cultured on or in type I collagen gel are induced to extend long cellular processes. This elongation of the processes occurs via integrin-binding to the sequence DGEA on type I collagen fibrils. Here the signal transduction mechanism, including phosphorylation, seems to be critical to induce and sustain the cellular process architecture. It is thus concluded that the multiple three-dimensional adhesive structures of hepatic stellate cells to the three-dimensional ECM participate in the development and sustaining of the three-dimensional cellular processes of the cells within the perisinusoidal space as well as of the cells *in vitro*.

Acknowledgments. Research conducted at the authors' laboratory cited in this review article, was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan.

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(*Received for publication, January 9, 2001*

and accepted, January 9, 2001)