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Human pathological basis of blood vessels and stromal tissue for nanotechnology☆

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ABSTRACT

The recent development of nanotechnology has already produced clinically applicable "nanodrugs," which are largely dependent on a novel concept for the drug delivery system. Thus the elucidation of local pharmacokinetics of nanodrugs is indispensable for the further development of nanomedicine; however, the detailed pathophysiology associated with nano-sized materials especially in pathologic lesions has not been well-described. In this review article, the microscopic appearance of vascular pericytes in addition to endothelial cells is discussed in the normal state and also in several pathological conditions which could be the major targets for nanomedicine. Moreover, the role of stromal tissue including myofibroblasts is also focused on, as well as inflammatory cells. Finally, the significance of disease-specific tissue structure in the establishment of personalized nanomedicine is discussed.

Contents

1. Introduction

The promising efficacy of nanomedicine is highly prescribed by the permeability and retention of nanodrugs within the target lesion, which is described as the enhanced permeability and retention (EPR) effect especially in solid tumor [1,2]. Most of the nanodrugs are delivered via blood flow, i.e. through blood vessels; therefore, the role of vasculatures in normal tissue as well as in pathologic lesions has been well-characterized including morphological abnormalities such as fenestration or defect of endothelial cells in solid tumor [3–5]. In addition, the involvement of vascular pericytes in the permeation of

Abbreviations: EPR, enhanced permeability and retention; CAFs, cancer associated fibroblasts; TAMs, tumor-associated macrophages; PDGFRβ, platelet-derived growth factor receptor beta; αSMA, alpha smooth muscle actin; TGFβ, transforming growth factor beta; 5-FU, 5-fluorouracil; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; TNF-α, tumor necrosis factor alpha; iNOS, inducible nitric oxide synthase; MHC, major histocompatibility complex; LDL, low-density lipoprotein.

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nanoparticles was recently reported [6]. However, detailed pathological observation of vascular elements in view of nanodrug-associated pathophysiology is still lacking, especially in human tissue.

In recent cancer research, the significance of a non-vascular microenvironment which consists of non-cancer cells such as cancer associated fibroblasts (CAFs) or tumor-associated macrophages (TAMs) has been recognized as a major factor for cancer promotion [7]. These stromal components could be possible players to modulate pharmacokinetics not only for small molecules but also for nanodrugs, because the stromal cells are usually located between the blood vessels and the pharmacological targets such as cancer cells. However, the pathophysiological approach for non-vascular stromal cells in nanomedicine has been insufficiently discussed.

This review summarizes and discusses the microscopic appearance of histological components, especially the blood vessels and stromal cells, in normal and major pathological conditions. Such aspects will give us idea regarding how to establish the tissue-type specific personalized nanomedicine.

2. Histopathology of blood vessels

Intravenously injected nanodrugs will be delivered via blood flow into the pathological lesion through arterioles (20 to 100 μm in diameter), and released from capillaries (7 to 8 μm), not from arteries or veins except in hemorrhagic conditions; thus, the principal vasculature for nanomedicine must be such smaller vessels, and the major players here are vascular endothelial cells and pericytes. The basic structure of arterioles and capillaries is shown in Fig. 1. Arterioles consist of endothelial cells, vascular smooth muscle cells and adventitia as well as larger muscular arteries, while capillaries lined by endothelial cells are supported on the outside by pericytes embedded in a thin basement membrane [8,9].

2.1. Endothelial cells

The entire vascular system is lined internally by a single layer of spindle-shaped endothelial cells. In human tissue, these cells can be identified immunohistochemically with antibodies against CD31, CD34 and factor VIII-related antigen (Fig. 2A, C) [9]. Their structural integrity is fundamental to maintain vessel wall homeostasis and permeability. Endothelial cells form junctional complexes by tight, adherens or gap junctions[10]. Claudin family proteins within tight junctions create the barrier and regulate electrical resistance between cells (Fig. 2B, D) [11,12]. Adherens junctions regulate permeability to soluble molecules and have a role in contact inhibition. Gap junctions consisting of connexin family proteins form channels between adjacent cells [13]. In association with nanodrug delivery, endothelial cells (1) serve as a semipermeable membrane, controlling the transfer of small and large molecules through the walls of arterioles and capillaries; (2) modulate vascular tone and blood flow which directly affect the accumulation of nanodrugs; (3) regulate inflammatory response controlling leukocyte interactions with vessel walls; and (4) modify lipoproteins such as oxidized LDL causing endothelial dysfunction in the artery wall [9].

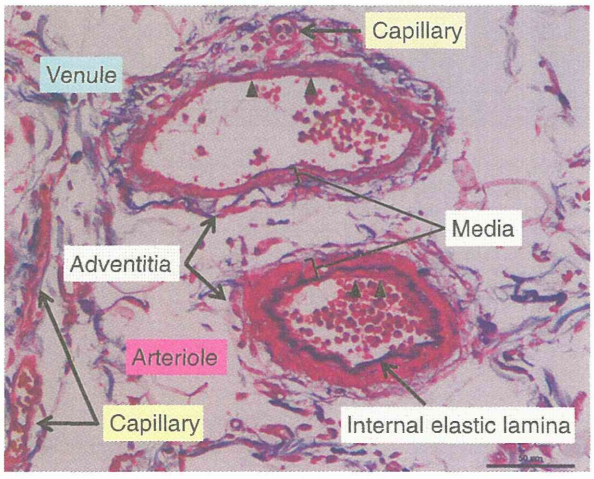

Fig. 1. Basic structure of blood vessels. Elastica–Masson staining of human duodenum is shown. The arteriole and venule consist of intima (arrow head), media and adventitia. Intimal elastic lamina can be identified in arteriole, while it is often ambiguous in venule. Arteriole consists of thick vascular smooth muscle layer (media), while media in venule are thin and often discontinuous. The capillary is composed of thin layer of mural cell. It is hard to identify endothelial cells by Elastica–Masson stain or Hematoxylin and Eosin stain in capillaries or even larger vessels. Scale bar is 50 μm.

2.2. Pericytes

Historically, pericytes were discovered and originally describe more than 100 years ago by scientists who were interested in the nature of capillary contractility [10]. The basic definition of mature pericytes as a mural cell embedded within the vascular basement membrane was made by electron microscopic observation in capillaries [10], although the term pericyte is frequently adopted to denote any microvascular periendothelial mesenchymal cells including vascular smooth muscle cells [11]. Currently, no pericyte-specific molecular markers have yet been identified, while several molecules expressed in murine pericytes, such as NG2, PDGFRβ and RGS-5, have been reported [11]. In general human pathology, the term pericyte is scarcely described, while the vascular smooth muscle cells have been well characterized [9], and established immunohistochemical markers for pericytes are limited. As shown in Fig. 2, αSMA, which is most reliable immunohistochemical marker for vascular smooth muscle cell (Fig. 2E), is also expressed in pericyte in capillary, while discontinuous or defective staining pattern is often observed (Fig. 2G). Anti-PDGFRβ antibody is another attractive candidate to identify pericytes in capillary, although myofibroblast adjacent to pericyte is also positive for PDGFRβ (Fig. 2H). In addition, mural cells in precapillary arteriole are positive for both PDGFRβ and αSMA (Fig. 2), thus it is hard to distinguish pericytes from vascular smooth muscle cells by immunohistochemistry. In human specimen, desmin is negative in vascular mural cells except in large muscular artery (Fig. 2F).

2.3. Pathologic condition of blood vessels

In diagnostic pathology, the alteration of vasculature in number and morphology is often recognized as a disease-specific phenomenon. In this section, the representative appearance of vascular alteration in cancerous tissue and inflammatory tissue including infectious diseases, which are major target diseases for nanomedicine, is presented.

2.3.1. Cancer

The EPR effect is now the central dogma for anticancer drug design using nanotechnology [1], which is based on the unique anatomical–pathophysiological nature of tumor blood vessels that facilitates transport of macromolecules into tumor tissues. Molecules larger than 40 kDa such as nanodrugs are selectively leaked out from tumor vessels, but not from normal vasculatures [14–18]; therefore, it is essential to comprehend the vascularity and the structure of tumor blood vessels in target cancerous lesion for nanomedicine. In practical surgical pathology, however, the morphological appearance and the number of vasculatures vary according to the organ or histological subtypes of tumor. In fact, even within the same histological subtype of "adenocarcinoma," the majority of ovarian cancer and colon cancer belong to the category of hypervascular tumor, while the scirrhous type of gastric cancer and pancreas cancer is usually categorized into hypovascular tumor. More importantly, the structure of tumor vasculature appears to be different according to each tissue. The αSMA-positive mural cells were discontinuously observed in tumor vessels of ovarian and colonic

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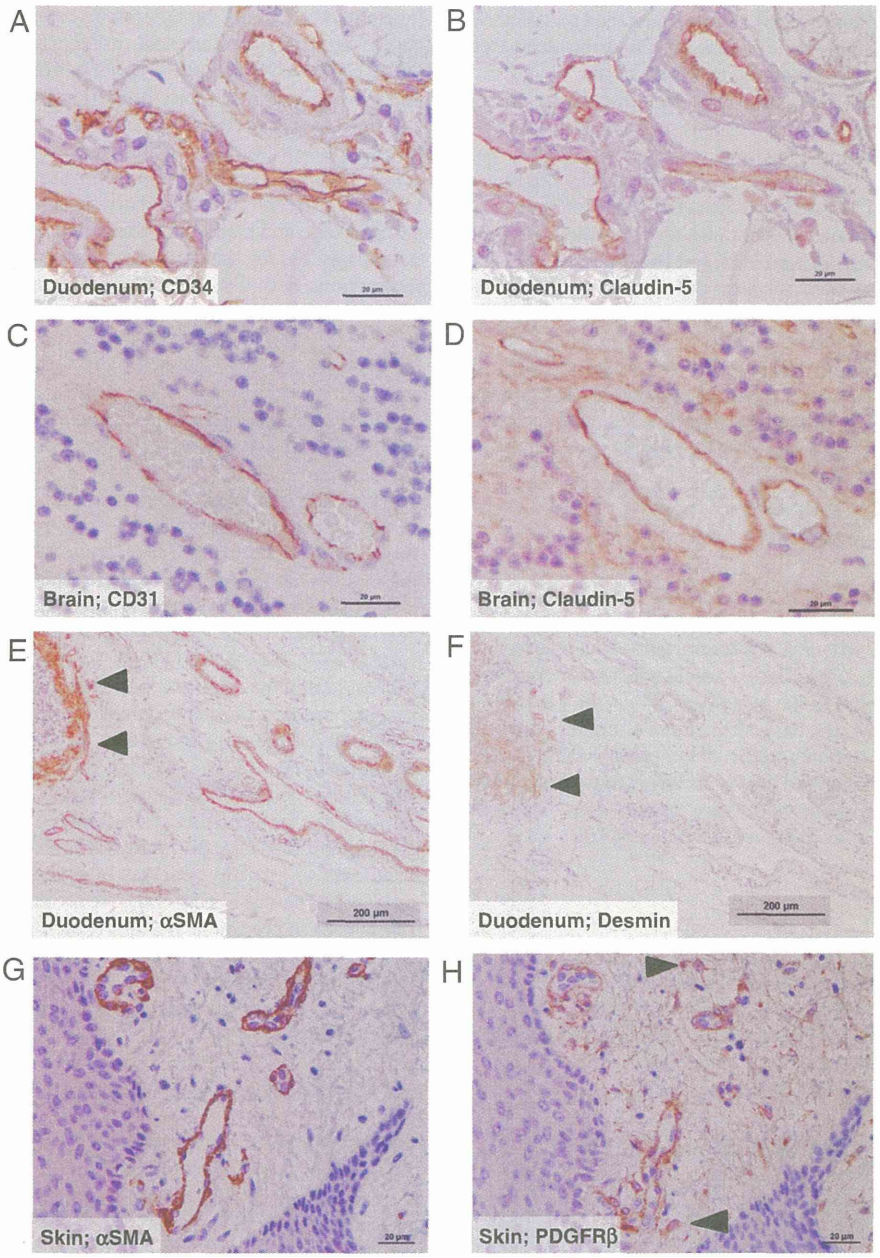

Fig. 2. Immunohistochemical appearance of vascular mural cells. The entire vascular system is lined internally by a single layer of spindle-shaped endothelial cells, which are identified immunohistochemically with antibodies against CD34 (A, duodenum) and CD31 (C, brain). Claudin-5 is immunopositive in endothelial cells (B, D), meaning the formation of tight junction barrier. Immunohistochemistry for αSMA visualizes vascular smooth muscle cells in large vessels such as arteries and veins as well as muscularis mucosa (E; duodenum, arrowhead), while the expression of Desmin is only detected in muscularis mucosa and not in vascular mural cells (F; duodenum, arrowhead). The pericytes in capillary are recognized by anti-αSMA antibody (G; skin), and anti-PDGFRβ antibody (H; skin) which also reacts upon myofibroblasts adjacent to vasculature (H, arrowhead). Antibodies employed here are as follows; anti-CD31 (Mouse Monoclonal clone:1A10, Novocastra), anti-CD34 (Mouse Monoclonal clone:QBEnd10, DAKO), Claudin-5 (Rabbit Polyclonal, Abcam), anti-αSMA (Mouse Monoclonal clone:1A4, Thermo Scientific), anti-PDGFRβ (Rabbit Monoclonal clone:C82A3, CST), and anti-Desmin (Mouse Monoclonal clone:D33, DAKO).

adenocarcinoma (Fig. 3A, B), while thick αSMA-positive pericytes entirely covered the endothelial cells in gastric adenocarcinoma (Fig. 3E, F) [19]. Moreover, the multilayered thick αSMA-positive pericytes form glomeruloid vessel in pancreatic adenocarcinoma (Fig. 3G. H). Currently, three types of structural variety of tumor blood vessel can be proposed; 1) thin, defective αSMA-positive pericyte coverage, 2) thin, αSMA-positive pericyte coverage, and 3) thick or glomeruloid, αSMA-positive pericyte coverage (Table 1); however, there is no established clinicopathological evidence for drug permeability and sensitivity in association with patients' outcome. In the murine experimental model of pancreatic ductal adenocarcinoma, the reduction of αSMA-positive pericytes by blocking TGFβ signaling increased accumulation of nanoparticles in tumor tissue [20,21], suggesting that the permeability of nanodrugs would be affected by the vascular density and also the structure of tumor vessels such as coverage of pericytes. In fact, glomeruloid microvascular proliferation, usually observed in glioblastoma and dominantly composed of αSMA-positive pericytes [22], was associated with lack of response to chemotherapy in breast

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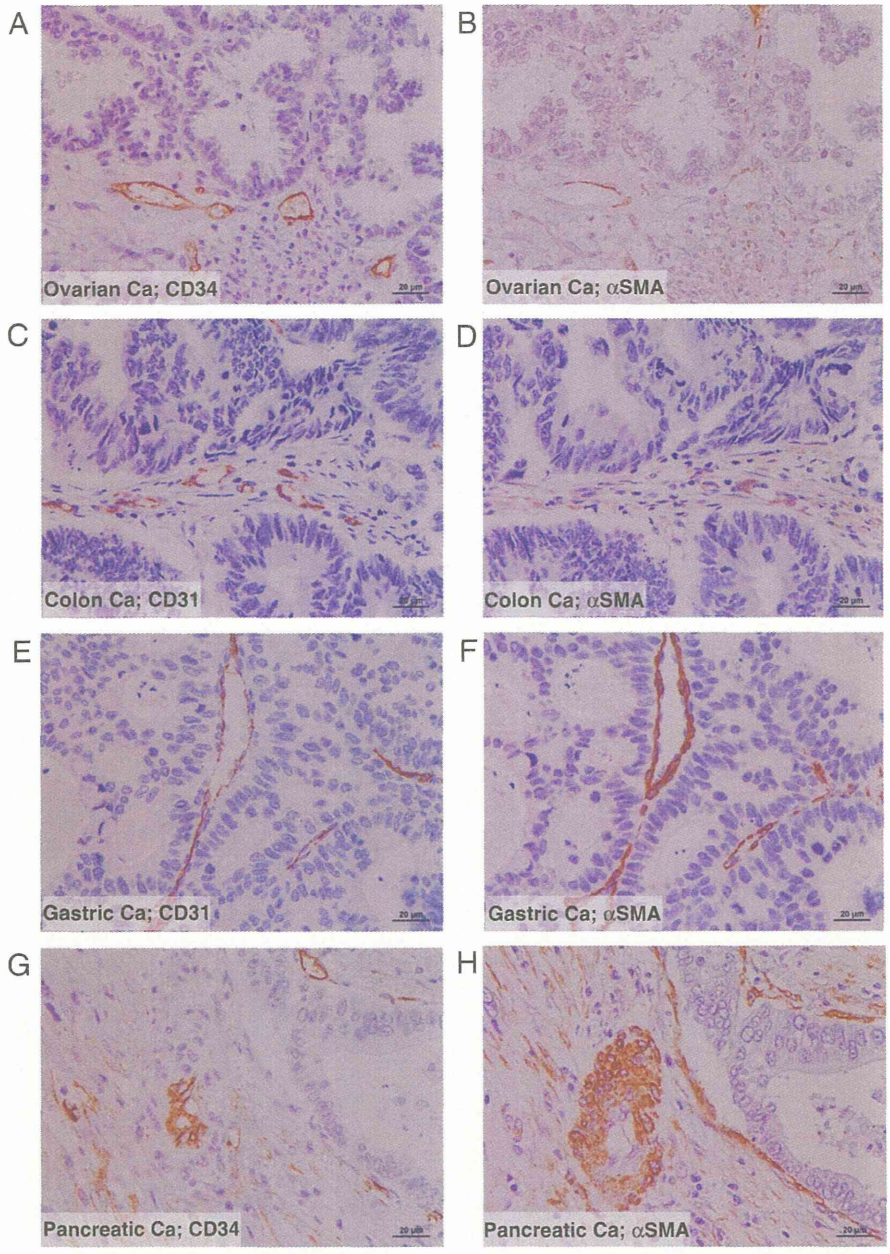

Fig. 3. Variation of tumor vasculature and expression of molecular markers. In ovarian serous papillary adenocarcinoma (Ovarian Ca) (A, B) and colonic tubular adenocarcinoma (Colon Ca) (C, D), anti-CD34 antibody (A) and anti-CD31 antibody (C) recognize endothelial cells, while αSMA-positive pericytes discontinuously cover capillaries (B, D). Gastric tubular adenocarcinoma (Gastric Ca) contains tumor associated vessels entirely covered with endothelial cells (E, anti-CD31) and αSMA-positive pericytes (F, anti-SMA). In pancreatic ductal adenocarcinoma (Pancreatic Ca), glomeruloid vessels with defective coverage of endothelial cells (G, anti-CD34) and multilayered plump αSMA-positive pericytes (H, anti-αSMA) are often observed.

cancer, although the patients were admitted with small molecular anti-cancer agents such as doxorubicin and 5-FU, not nano-sized drugs [23]. Altogether, type 3), thick or glomeruloid, αSMA-positive pericyte coverage in tumor blood vessel, seems to be associated with lower drug sensitivity and patients' poor prognosis in various types of cancer [24–26]. In addition to the experimental model using endothelial cells and also pericytes, mass clinicopathological analysis based on the three structural patterns of tumor vasculature will provide us with the

Table 1
Structural varieties of blood vessel (pericyte coverage).

Type	Thickness	Coverage	Representative cancer	Response for chemotherapy
1	Thin	Defective	Ovarian, colonic adenocarcinoma	Good
2	Thin	Continuous	Almost all types of cancer	Intermediate
3	Thick	Glomeruloid	Pancreatic ductal adenocarcinoma, Glioblastoma	Poor

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evidence that vascular structure is one of the prominent factors to determine the drug efficacy and also the patients' prognosis.

2.3.2. Inflammatory and infectious disease

In the early phase of inflammatory condition induced by injury or infection, endothelial dysfunction rapidly occurred within minutes by histamine, serotonin and other vasoactive mediators that induce excessive vascular permeability [9]. After several hours or even days, endothelial activation will be induced by cytokines and bacterial products which cause inflammatory injury and septic shock. Such reversible changes in the functional state of endothelial cells are invisible by histological examinations, while the neovascularization of capillaries discontinuously covered with αSMA-positive pericytes is microscopically observed in inflammatory granulation tissue (Fig. 4A, B). Moreover, vascular remodeling is often observed in the later phase of specific inflammatory condition such as wound healing process or prolonged latent infection [27]. The pulmonary infection by *Mycobacterium tuberculosis* forms caseous necrosis with Langerhans cells, and sometimes causes

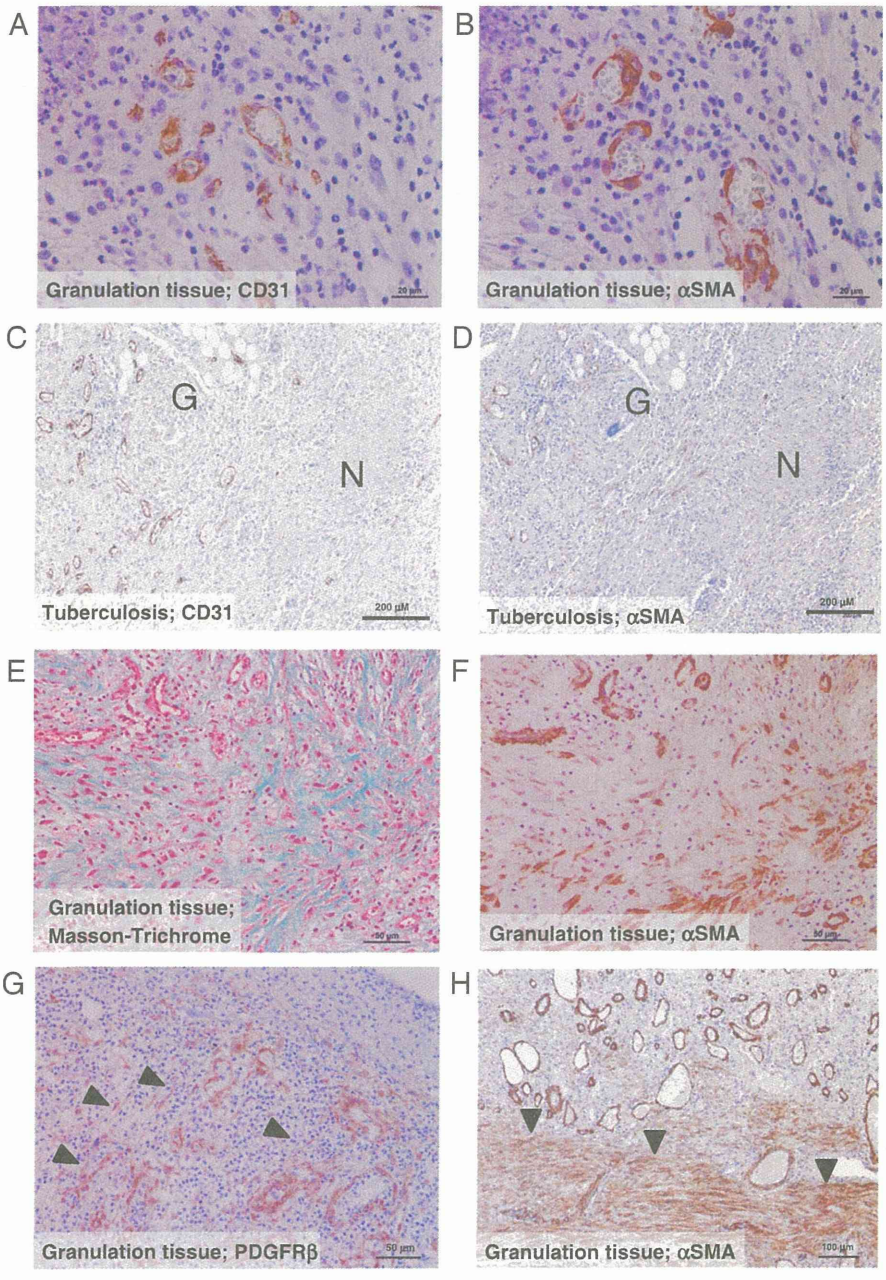

Fig. 4. Vasculatures and stromal components in inflammatory and infectious condition. A, B: The neovascularization of capillary discontinuously covered with endothelial cells (A, anti-CD31) and αSMA-positive pericytes (B, anti-αSMA) was microscopically observed in edematous granulation tissue. C, D: *Mycobacterium tuberculosis* formed caseous necrosis (N) and epithelioid granuloma with Langerhans cells in the lung (G). Around this lesion, vascular proliferation was observed (C, anti-CD31), and the vessels were covered with αSMA-positive pericytes (D, anti-αSMA), which was similar to type 3 tumor vasculature. Within necrotic area, neovascularization was not observed, meaning the derogation of drug delivery (C). E, F, G, H: The proliferative granulation tissue of skin ulcer is shown. The spindle cells with muscular elements such as vascular smooth muscle cell and myofibroblasts were stained in red by Masson-Trichrome (E), and immunohistochemically reacted upon anti-αSMA antibody (F). The long axis of PDGFRβ-positive fibroblasts in the exudative layer is perpendicular to the surface (G, arrowheads), while αSMA-positive myofibroblasts arrange parallel to the surface in cicatrizing layers (H, arrowheads). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

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cavity formation in the prolonged or latent phase [9]. The majority of the vasculature around the caseous necrosis is covered with thick αSMA-positive pericytes (Fig. 4C, D), which is similar to the type 3) tumor vasculature discussed in Section 2.3.1, suggesting the low leakage of macromolecules even if endothelial activation was induced by bacterial products or cytokines. In addition, within necrotic area, neovascularization is not prominent, which means the derogation of drug delivery (Fig. 4C). Currently, a histopathological approach for vascular structure in inflammatory and infectious conditions is still being investigated to establish the new therapeutic strategy using nanodrugs against such diseases.

3. Histopathology of stromal tissue

Stromal tissue other than vasculature consists of connective collagenous matrix, fibrotic cells including myofibroblasts and inflammatory cells. The barrier to successful nanodrug delivery after leakage from vasculature to target cells should be such stromal tissue. Tumor cells are often surrounded by coagulation-derived matrix gel such as fibrin gel, although macromolecules can diffuse across the matrix tissue for a considerable distance; e.g., a 160 kDa protein, IgG, can freely diffuse across 5 mm overnight in 1 % agarose gel [1], meaning that the thickness of the matrix would not seriously affect the nanodrug delivery. In this section, myofibroblasts and inflammatory cells, the major cellular components of stromal tissue, are discussed in terms of nanodrug distribution.

3.1. Myofibroblasts

The myofibroblasts, which usually appear spindle, and often stellate with long cytoplasmic extensions, were originally identified within experimental granulation tissue by electron micrographs [28], and share morphologic features common with those of fibroblasts and smooth muscle cells. In ultrastructural observations, myofibroblasts contain numerous bundles of cytoplasmic microfilaments (stress fibers) [29], and the cells are connected by intermediate or adherens junction [30].

Immunohistochemically, the stress fibers in myofibroblasts react with anti-αSMA antibody (Fig. 4F), and desmin is another muscle differentiation marker for myofibroblasts as well as vimentin, but these are not specific for myofibroblasts [29]. Myofibroblasts can be observed in multiple normal human tissues including any types of mucosal epithelium [31–34], lung [35], bone marrow[36] and many other organs [29]. In granulation tissue, PDGFRβ-positive fibroblasts are mostly found in the exudative layer, and the long axis is perpendicular to the surface (Fig. 4G), whereas in the exudative-productive and cicatrizing layers, the long axis of αSMA-positive myofibroblasts is parallel to the surface (Fig. 4H) [37]. These data suggest that the spatial orientation of myofibroblasts in granulation tissue varies, possibly to maximize the transmission of contractile forces and thereby affect wound closure [29]. The biochemical functions of myofibroblasts are not only for contraction, but also synthetic properties for several extracellular matrix components: collagen type I, III, IV and V [38–41], glycoproteins [42] and proteoglycans such as fibronectin [43].

Currently, there are several hypotheses about the origin of myofibroblasts, including resting fibroblasts, fibrocytes, epithelial cells following epithelial–mesenchymal transition, endothelial cells following endothelial–mesenchymal transition, and pericytes [8,44,45], although the origin of myofibroblasts is far from being elucidated.

3.2. Inflammatory cells

The inflammatory response occurs in stromal tissue including circulating cells, and cellular and extracellular constituents of connective tissue. The circulating cells include neutrophils, monocytes, eosinophils, lymphocytes, and basophils, while the connective tissue cells consist of mast cells, fibroblasts and resident macrophages [9]. Phagocytosis by neutrophils and macrophages is one of the fundamental protective responses against infection and foreign bodies including certain therapeutic agents. Thus the phagocytes, e.g., macrophages in atherosclerosis, are now a novel target for nanomedicine[46]. In human pathological specimens, macrophages can be immunohistochemically identified with anti-CD68 antibody.

3.3. Pathologic condition of stromal tissue; cancer stroma

The cancer stroma, which consists of non-cancer cells such as fibroblasts including myofibroblasts, inflammatory cells and vasculatures, has been recently recognized as a major factor for cancer promotion. Especially, cancer associated fibroblasts (CAFs), which are identified by the expression of actin, promote the proliferation and progression of cancer through the production of growth factors, angiogenetic factors and metalloproteinases, allowing invasion and metastasis of cancer cells [7]. CAFs appear to originate from resident fibroblasts [47,48] and other mesenchymal cells including pericytes and stellate cells [49]. Immunohistochemical analysis revealed the proportions of stromal αSMA-, NG2-, PDGFRβ-, FAP-, FSP- and TN-C-positive myofibroblasts within mice xenografts [50], while the expression of these molecular markers according to the cancer types or organs has not been confirmed especially in human specimens. In fact, the expression pattern of these molecular markers in cancer stromal cells was varied according to the histological type or site of organ. In pancreas adenocarcinoma which usually represents strong desmoplastic reaction of dense fibrotic tissue [51], stromal cells were positive for αSMA and/or PDGFRβ, although it was varied in each individual cases; even if the histological subtype of adenocarcinoma was similar (Fig. 5). Moreover, the amount of PDGFRβ-positive -, not αSMA-positive-, stromal cells was statistically correlated with poor prognosis of the patients with pancreatic ductal adenocarcinoma [52], suggesting that molecular expression in stromal cells might be associated with the tumor progression and/or drug sensitivity in pancreatic cancer. Currently, the following histological subtypes of cancer stroma can be supposed (Table 2).; 1) sparse desmoplasia (Fig. 5A, B), 2) dense desmoplasia with PDGFRβ expression (Fig. 5C, D), 3) dense desmoplasia with αSMA expression (Fig. 5E, F), and 4) scar-like desmoplasia composed of scattered αSMA-positive cells and collagen bundles (Fig. 5G, H). Type 1) is sometimes observed in ovarian adenocarcinoma and early stages of gastric and colorectal adenocarcinoma, thus the association with favorable patients' prognosis, while 2) to 4) tend to be found in pancreatic ductal adenocarcinoma or scirrhous type of gastric adenocarcinoma, meaning the poorer patients' outcome. Considering the strategies for nanomedicine-mediated cancer treatment according to the type of cancer stroma, dense and scar-like desmoplasia (type 2, 3 and 4) can induce stromal stiffness resulting in abrogation of nanodrug delivery, even though nanodrugs are selectively leaked out from tumor vessels by the EPR effect. An excess in lysyl oxidase (LOX) activity has been correlated with stromal stiffness and poor prognosis in several types of cancer [53,54], although there are conflicting views on the use of LOX inhibitor in cancer treatment [53]. Extracellular matrix deposition and increased stromal stiffness have been noted to enhance tumor progression through altering integrin signaling and focal adhesions [55]. The activation and clustering of integrins dependent on stromal stiffness lead to a mechanosensitive signaling cascade [56], and targeting αVβ3-integrin has been shown to inhibit bone metastasis of breast cancer cells in mice bearing mammary adenocarcinoma [57]. The therapies targeting cancer stroma including CAFs and related molecules, such as indicated above, could be combined with nanomedicine-mediated cancer treatment to concur the stromal barrier against nanodrugs.

Inflammatory cells such as macrophages, neutrophils and lymphocytes are other major players in cancer stroma affecting the tumor promotion. Tumor-associated macrophages (TAMs) and neutrophils are considered as stimulators of tumor progression and angiogenesis

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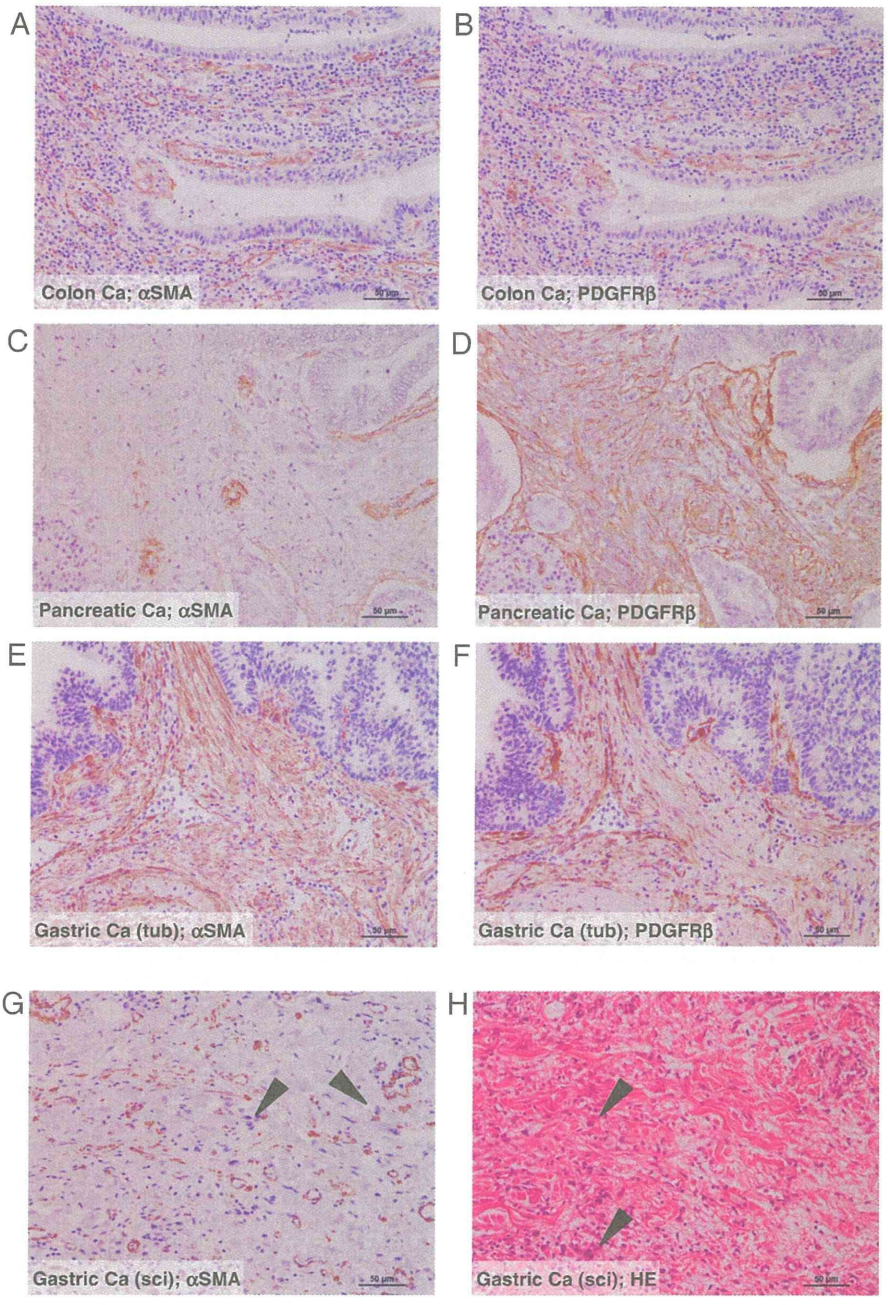

Fig. 5. Stromal variation in cancer tissue. Human surgical specimens were immunostained with antibodies against αSMA (A, C, E, G) and PDGFRβ (B, D, F). A, B: In the case of colonic tubular adenocarcinoma (Colon Ca), vascular pericytes were immunopositive for αSMA and also for PDGFRβ, and cancer stroma was composed of majority of inflammatory cells and negligible αSMA-positive myofibroblasts. C, D: A case of pancreatic ductal adenocarcinoma (Pancreatic Ca) revealed dense desmoplastic reaction in which spindle-shaped stromal cells were negative for αSMA (C) but positive for PDGFRβ (D). E, F, G, H: In gastric adenocarcinoma, αSMA- and PDGFRβ-positive dense stromal cells were observed in well differentiated tubular adenocarcinoma (Gastric Ca (tub)) (E, F), while scar-like dense collagen bundle and scattered tumor cells (arrowhead) were exhibited in scirrhous type-poorly differentiated adenocarcinoma (Gastric Ca (sci)) (G, H; Hematoxylin & Eosin staining (HE)).

via production of multiple cytokines such as VEGF, HGF and IL-8 [7]. Two distinct states of polarized activation for macrophages have been advocated: the classically activated (M1) macrophages and the alternatively activated (M2) macrophages [58–60]. M1 macrophages express a series of proinflammatory cytokines, chemokines and effector molecules such as IL-12, TNF-α, iNOS and MHCI/II, while M2 macrophages express a wide array of anti-inflammatory molecules such as IL-10 and arginase [61]. In human specimens, two types of macrophages, M1 and M2, are positive for anti-CD68 antibody, while M2 macrophages can be identified with anti-CD163 antibody. The role of TAM in tumors is still controversial. In colorectal cancers, TAMs are proinflammatory like M1, and play an antitumor role, which leads to a good prognosis [62,63]. However, in most tumors such as breast, prostate, uterine and lung cancer, TAMs are considered to be anti-inflammatory, i.e., the M2 type, and correlated with a poor prognosis [64]. TAMs orchestrate various aspects of cancer promotion by affecting angiogenesis, immunomodulation, matrix deposition and remodeling [65]. In addition, phagocytosis, another indispensable function of macrophages, must be considered for nanodrug efficacy, because the nanocarriers must be hidden from the reticulo-endothelial system, which destroys

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Table 2
Histological subtype of cancer stroma.

Type	Desmoplasia	PDGFRβ	αSMA	Representative cancer	Response for chemotherapy
1	Absent	−	−	Early stage of colonic, gastric adenocarcinoma	Good
2	Dense fibrosis	+	−	Majority of cancer	Various, relatively poor
3	Dense fibrosis	+	+		Various, relatively poor
4	Scar like	−	±	Scirrhous type of gastric adenocarcinoma	Poor

any foreign material though opsonization followed by phagocytosis [66–68]. Further pharmacokinetic and clinicopathological analyses for nanodrugs in association with type and amount of TAMs in human cancer specimens are required for the development of nanomedicine.

4. Conclusion

Nanomedicine is the application of nanotechnology in monitoring, diagnosing, preventing, repairing or curing diseases and damaged tissues in the biological system, and the nanomedicine approach will help to establish patient-specific personalized medicine in the near future [69]. The promising efficacy of nanomedicine is highly prescribed by the permeability and retention of nanodrugs within the target lesion such as the EPR effect; therefore, it is extremely important to comprehend the vascular and stromal structures in the target lesion. However, as shown in this review, the cancerous tissues, even if they are in the same organ or have the same histological subtype, consist of various types of tumor vessels and also stromal patterns. Moreover, it is no exaggeration to say that almost all diseases other than cancer have never been analyzed in respect to their vascular and stromal structures for the nanomedicine approach. Pathological examination to evaluate the vascular and stromal patterns in the target lesion is required before the development or administration of nanodrugs. In addition, the establishment of nano-pathophysiology is necessary to understand the disease-specific and/or patient-specific pharmacokinetics of nanodrugs, which will lead to the promising efficacy of nanomedicine and establish personalized nanomedicine.

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References

[1] J. Fang, H. Nakamura, H. Maeda, The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect, Adv. Drug Deliv. Rev. 63 (2011) 136–151.
[2] V. Torchilin, Tumor delivery of macromolecular drugs based on the EPR effect, Adv. Drug Deliv. Rev. 63 (2011) 131–135.
[3] J. Daruwalla, K. Greish, C. Malcontenti-Wilson, V. Muralidharan, A. Iyer, H. Maeda, C. Christophi, Styrene maleic acid-pirarubicin disrupts tumor microcirculation and enhances the permeability of colorectal liver metastases, J. Vasc. Res. 46 (2009) 218–228.
[4] H. Maeda, J. Fang, T. Inutsuka, Y. Kitamoto, Vascular permeability enhancement in solid tumor: various factors, mechanisms involved and its implications, Int. Immunopharmacol. 3 (2003) 319–328.
[5] S.A. Skinner, P.J. Tutton, P.E. O'Brien, Microvascular architecture of experimental colon tumors in the rat, Cancer Res. 50 (1990) 2411–2417.
[6] M.R. Kano, Y. Komuta, C. Iwata, M. Oka, Y.T. Shirai, Y. Morishita, Y. Ouchi, K. Kataoka, K. Miyazono, Comparison of the effects of the kinase inhibitors imatinib, sorafenib, and transforming growth factor-beta receptor inhibitor on extravasation of nanoparticles from neovasculature, Cancer Sci. 100 (2009) 173–180.
[7] H. Li, X. Fan, J. Houghton, Tumor microenvironment: the role of the tumor stroma in cancer, J. Cell. Biochem. 101 (2007) 805–815.
[8] A. Armulik, G. Genove, C. Betsholtz, Pericytes: developmental, physiological, and pathological perspectives, problems, and promises, Dev. Cell 21 (2011) 193–215.
[9] R.S. Cotran, Pathologic Basis of Disease, 6th ed. Saunders, 1999.
[10] G. Bazzoni, E. Dejana, Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis, Physiol. Rev. 84 (2004) 869–901.
[11] C.M. Van Itallie, J.M. Anderson, The molecular physiology of tight junction pores, Physiology (Bethesda) 19 (2004) 331–338.
[12] N. Sawada, M. Murata, K. Kikuchi, M. Osanai, H. Tobioka, T. Kojima, H. Chiba, Tight junctions and human diseases, Med. Electron Microsc. 36 (2003) 147–156.
[13] G. Sohl, K. Willecke, Gap junctions and the connexin protein family, Cardiovasc. Res. 62 (2004) 228–232.
[14] Y. Matsumura, H. Maeda, A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs, Cancer Res. 46 (1986) 6387–6392.
[15] H. Maeda, The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting, Adv. Enzyme Regul. 41 (2001) 189–207.
[16] H. Maeda, SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy, Adv. Drug Deliv. Rev. 46 (2001) 169–185.
[17] J. Fang, T. Sawa, H. Maeda, Factors and mechanism of "EPR" effect and the enhanced antitumor effects of macromolecular drugs including SMANCS, Adv. Exp. Med. Biol. 519 (2003) 29–49.
[18] H. Maeda, G.Y. Bharate, J. Daruwalla, Polymeric drugs for efficient tumor-targeted drug delivery based on -effect, Eur. J. Pharm. Biopharm. 71 (2009) 409–419.
[19] L. Zhang, H. Nishihara, M.R. Kano, Pericyte-coverage of human tumor vasculature and nanoparticle permeability, Biol. Pharm. Bull. 35 (2012) 761–766.
[20] M.R. Kano, Y. Bae, C. Iwata, Y. Morishita, M. Yashiro, M. Oka, T. Fujii, A. Komuro, K. Kiyono, M. Kaminishi, K. Hirakawa, Y. Ouchi, N. Nishiyama, K. Kataoka, K. Miyazono, Improvement of cancer-targeting therapy, using nanocarriers for intractable solid tumors by inhibition of TGF-beta signaling, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3460–3465.
[21] H. Cabral, Y. Matsumoto, K. Mizuno, Q. Chen, M. Murakami, M. Kimura, Y. Terada, M.R. Kano, K. Miyazono, M. Uesaka, N. Nishiyama, K. Kataoka, Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size, Nat. Nanotechnol. 6 (2011) 815–823.
[22] A.M. Rojiani, K. Dorovini-Zis, Glomeruloid vascular structures in glioblastoma multiforme: an immunohistochemical and ultrastructural study, J. Neurosurg. 85 (1996) 1078–1084.
[23] L.A. Akslen, O. Straume, S. Geisler, T. Sorlie, J.T. Chi, T. Aas, A.L. Borresen-Dale, P.E. Lonning, Glomeruloid microvascular proliferation is associated with lack of response to chemotherapy in breast cancer, Br. J. Cancer 105 (2011) 9–12.
[24] O. Straume, P.O. Chappuis, H.B. Salvesen, O.J. Halvorsen, S.A. Haukaas, J.R. Goffin, L.R. Begin, W.D. Foulkes, L.A. Akslen, Prognostic importance of glomeruloid microvascular proliferation indicates an aggressive angiogenic phenotype in human cancers, Cancer Res. 62 (2002) 6808–6811.
[25] F. Tanaka, H. Oyanagi, K. Takenaka, S. Ishikawa, K. Yanagihara, R. Miyahara, Y. Kawano, M. Li, Y. Otake, H. Wada, Glomeruloid microvascular proliferation is superior to intratumoral microvessel density as a prognostic marker in non-small cell lung cancer, Cancer Res. 63 (2003) 6791–6794.
[26] W.D. Foulkes, J.S. Brunet, I.M. Stefansson, O. Straume, P.O. Chappuis, L.R. Begin, N. Hamel, J.R. Goffin, N. Wong, M. Trudel, L. Kapusta, P. Porter, L.A. Akslen, The prognostic implication of the basal-like (cyclin E high/p27 low/p53+/glomeruloid-microvascular-proliferation+) phenotype of BRCA1-related breast cancer, Cancer Res. 64 (2004) 830–835.
[27] B.M. Dulmovits, I.M. Herman, Microvascular remodeling and wound healing: a role for pericytes, Int. J. Biochem. Cell Biol. 44 (2012) 1800–1812.
[28] G. Gabbiani, G.B. Ryan, G. Majne, Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction, Experientia 27 (1971) 549–550.
[29] S.E. Mills, Histology for Pathologists, 3rd ed. Lippincott Williams & Wilkins, 2007.
[30] B. Hinz, G. Gabbiani, Cell-matrix and cell–cell contacts of myofibroblasts: role in connective tissue remodeling, Thromb. Haemost. 90 (2003) 993–1002.
[31] J. Boya, A.L. Carbonell, A. Martinez, Myofibroblasts in human palatal mucosa, Acta Anat. (Basel) 131 (1988) 161–165.
[32] F.H. Guldner, J.R. Wolff, D.G. Keyserlingk, Fibroblasts as a part of the contractile system in duodenal villi of rat, Z. Zellforsch. Mikrosk. Anat. 135 (1972) 349–360.
[33] G.I. Kaye, N. Lane, R.R. Pascal, Colonic pericryptal fibroblast sheath: replication, migration, and cytodifferentiation of a mesenchymal cell system in adult tissue. II. Fine structural aspects of normal rabbit and human colon, Gastroenterology 54 (1968) 852–865.
[34] A.P. Sappino, P.Y. Dietrich, O. Skalli, S. Widgren, G. Gabbiani, Colonic pericryptal fibroblasts. Differentiation pattern in embryogenesis and phenotypic modulation in epithelial proliferative lesions, Virchows Arch A Pathol Anat Histopathol 415 (1989) 551–557.
[35] Y. Kapanci, A. Assimacopoulos, C. Irle, A. Zwahlen, G. Gabbiani, "Contractile interstitial cells" in pulmonary alveolar septa: a possible regulator of ventilation–perfusion ratio? Ultrastructural, immunofluorescence, and in vitro studies, J. Cell Biol. 60 (1974) 375–392.

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[36] P. Charbord, H. Lerat, I. Newton, E. Tamayo, A.M. Gown, J.W. Singer, P. Herve, The cytoskeleton of stromal cells from human bone marrow cultures resembles that of cultured smooth muscle cells, Exp. Hematol. 18 (1990) 276–282.
[37] T. Kondo, Y. Ishida, Molecular pathology of wound healing, Forensic Sci. Int. 203 (2010) 93–98.
[38] K. Zhang, M.D. Rekhter, D. Gordon, S.H. Phan, Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. A combined immunohistochemical and in situ hybridization study, Am. J. Pathol. 145 (1994) 114–125.
[39] G. Gabbiani, M. Le Lous, A.J. Bailey, S. Bazin, A. Delaunay, Collagen and myofibroblasts of granulation tissue. A chemical, ultrastructural and immunologic study, Virchows Arch. B Cell Pathol. 21 (1976) 133–145.
[40] R. Rudolph, S. Guber, M. Suzuki, M. Woodward, The life cycle of the myofibroblast, Surg. Gynecol. Obstet. 145 (1977) 389–394.
[41] S.H. Barsky, C.N. Rao, G.R. Grotendorst, L.A. Liotta, Increased content of Type V Collagen in desmoplasia of human breast carcinoma, Am. J. Pathol. 108 (1982) 276–283.
[42] A.M. Gressner, M.G. Bachem, Cellular sources of noncollagenous matrix proteins: role of fat-storing cells in fibrogenesis, Semin. Liver Dis. 10 (1990) 30–46.
[43] A. Berndt, H. Kosmehl, D. Katenkamp, V. Tauchmann, Appearance of the myofibroblastic phenotype in Dupuytren's disease is associated with a fibronectin, laminin, collagen type IV and tenascin extracellular matrix, Pathobiology 62 (1994) 55–58.
[44] D.W. Powell, I.V. Pinchuk, J.I. Saada, X. Chen, R.C. Mifflin, Mesenchymal cells of the intestinal lamina propria, Annu. Rev. Physiol. 73 (2011) 213–237.
[45] J.A. Varga, M. Trojanowska, Fibrosis in systemic sclerosis, Rheum. Dis. Clin. North Am. 34 (2008) 115–143(vii).
[46] C. Psarros, R. Lee, M. Margaritis, C. Antoniades, Nanomedicine for the prevention, treatment and imaging of atherosclerosis, Maturitas 73 (2012) 52–60.
[47] N. Erez, M. Truitt, P. Olson, S.T. Arron, D. Hanahan, Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-kappaB-dependent manner, Cancer Cell 17 (2010) 135–147.
[48] B. Hinz, S.H. Phan, V.J. Thannickal, A. Galli, M.L. Bochaton-Piallat, G. Gabbiani, The myofibroblast: one function, multiple origins, Am. J. Pathol. 170 (2007) 1807–1816.
[49] U.M. Polanska, A. Orimo, Carcinoma-associated fibroblasts: non-neoplastic tumour-promoting mesenchymal cells, J Cell Physiol 228 (2013) 1651–1657.
[50] H. Sugimoto, T.M. Mundel, M.W. Kieran, R. Kalluri, Identification of fibroblast heterogeneity in the tumor microenvironment, Cancer Biol. Ther. 5 (2006) 1640–1646.
[51] R. Kalluri, M. Zeisberg, Fibroblasts in cancer, Nat. Rev. Cancer 6 (2006) 392–401.
[52] S. Yuzawa, M.R. Kano, T. Einama, H. Nishihara, PDGFRbeta expression in tumor stroma of pancreatic adenocarcinoma as a reliable prognostic marker, Med. Oncol. 29 (2012) 2824–2830.
[53] H.E. Barker, T.R. Cox, J.T. Erler, The rationale for targeting the LOX family in cancer, Nat. Rev. Cancer 12 (2012) 540–552.
[54] K.R. Levental, H. Yu, L. Kass, J.N. Lakins, M. Egeblad, J.T. Erler, S.F. Fong, K. Csiszar, A. Giaccia, W. Weninger, M. Yamauchi, D.L. Gasser, V.M. Weaver, Matrix crosslinking forces tumor progression by enhancing integrin signaling, Cell 139 (2009) 891–906.
[55] M.S. Samuel, J.I. Lopez, E.J. McGhee, D.R. Croft, D. Strachan, P. Timpson, J. Munro, E. Schroder, J. Zhou, V.G. Brunton, N. Barker, H. Clevers, O.J. Sansom, K.I. Anderson, V.M. Weaver, M.F. Olson, Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation to induce epidermal hyperplasia and tumor growth, Cancer Cell 19 (2011) 776–791.
[56] N.E. Sounni, A. Noel, Targeting the tumor microenvironment for cancer therapy, Clin. Chem. 59 (2013) 85–93.
[57] T. Bauerle, D. Komljenovic, M. Merz, M.R. Berger, S.L. Goodman, W. Semmler, Cilengitide inhibits progression of experimental breast cancer bone metastases as imaged noninvasively using VCT, MRI and DCE-MRI in a longitudinal in vivo study, Int. J. Cancer 128 (2011) 2453–2462.
[58] P.J. Murray, T.A. Wynn, Obstacles and opportunities for understanding macrophage polarization, J. Leukoc. Biol. 89 (2011) 557–563.
[59] M. Stein, S. Keshav, N. Harris, S. Gordon, Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation, J. Exp. Med. 176 (1992) 287–292.
[60] S. Goerdt, C.E. Orfanos, Other functions, other genes: alternative activation of antigen-presenting cells, Immunity 10 (1999) 137–142.
[61] A. Mantovani, S. Sozzani, M. Locati, P. Allavena, A. Sica, Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes, Trends Immunol. 23 (2002) 549–555.
[62] S.M. Ong, Y.C. Tan, O. Beretta, D. Jiang, W.H. Yeap, J.J. Tai, W.C. Wong, H. Yang, H. Schwarz, K.H. Lim, P.K. Koh, K.L. Ling, S.C. Wong, Macrophages in human colorectal cancer are pro-inflammatory and prime T cells towards an anti-tumour type-1 inflammatory response, Eur. J. Immunol. 42 (2012) 89–100.
[63] P. Dumont, A. Berton, N. Nagy, F. Sandras, S. Tinton, P. Demetter, F. Mascart, A. Allaoui, C. Decaestecker, I. Salmon, Expression of galectin-3 in the tumor immune response in colon cancer, Lab. Invest. 88 (2008) 896–906.
[64] A. Nardin, J.P. Abastado, Macrophages and cancer, Front. Biosci. 13 (2008) 3494–3505.
[65] N.B. Hao, M.H. Lu, Y.H. Fan, Y.L. Cao, Z.R. Zhang, S.M. Yang, Macrophages in tumor microenvironments and the progression of tumors, Clin. Dev. Immunol. 2012 (2012) 948098.
[66] F. Danhier, O. Feron, V. Preat, To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery, J. Control. Release 148 (2010) 135–146.
[67] Y. Malam, M. Loizidou, A.M. Seifalian, Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer, Trends Pharmacol. Sci. 30 (2009) 592–599.
[68] E. Gullotti, Y. Yeo, Extracellularly activated nanocarriers: a new paradigm of tumor targeted drug delivery, Mol. Pharm. 6 (2009) 1041–1051.
[69] A.S. Gupta, Nanomedicine approaches in vascular disease: a review, Nanomedicine 7 (2011) 763–779.

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