2. Imaging the pancreatic ECM

Palamadai N. Venkatasubramanian

Center for Basic M.R. Research, NorthShore University HealthSystem, Evanston, IL 60201, USA

Introduction

The extracellular matrix (ECM) is the non-cellular component present within all tissues and organs and provides essential physical scaffolding for the cellular constituents in addition to initiating crucial biochemical and biomechanical cues that are required for tissue morphogenesis, differentiation and homeostasis [1]. The ECM is essentially composed of water, proteins, and polysaccharides. However, the composition and topology of ECM in each tissue is unique and is generated during tissue development through a dynamic interaction between the various cellular components and the evolving cellular and protein microenvironment. The physical and biochemical characteristics of the ECM generate the biochemical and mechanical properties of each organ, such as its tensile and compressive strength and elasticity. The ECM also mediates protection by a buffering action that maintains extracellular homeostasis and water retention. Further, the ECM directs essential morphological organization and physiological function by binding growth factors and interacting with cell-surface receptors to transduce signal and regulate gene transcription. Cell adhesion to the ECM
is mediated by ECM receptors, such as integrins. Adhesion mediates cytoskeletal coupling to the ECM and is involved in cell migration through the ECM. The ECM is a highly dynamic structure that is constantly being remodeled. The properties of ECM vary not only from one tissue to another, but also from one physiological state to another, such as normal versus cancerous. However, the value of ECM changes induced by or related to pathology has yet to be fully targeted by imaging technology as a means to diagnose cancer and/or evaluate novel therapies.

**ECM composition**

The ECM is composed of two main classes of macromolecules: fibrous proteins and proteoglycans [1-3]. The main fibrous proteins are collagens, elastins, fibronectins and laminins [4]. Collagen is the most abundant fibrous protein within the ECM and constitutes up to 30% of the total protein mass. This scaffolding protein contributes to the tensile strength of tissue, regulates cell adhesion, supports chemotaxis and migration, and directs tissue development [1,5]. The main ECM component of interstitial tissue is fibrillar type I collagen. Type I collagen is the prototypical fibrillar collagen that represents up to 90% of the protein content of connective tissues. The bulk of interstitial collagen is transcribed and secreted by fibroblasts that either reside in the stroma or are from neighboring tissues. Type I collagen consists of two α1(I) chains and one α2(I) chain. The chains wrap around one another in a rope-like fashion to form the triple helix, nucleated at the N-terminus. Collagen fibrils are strengthened by covalent crosslinking and deamination by the enzyme lysyl oxidase. Type IV collagen, a non-fibrillar collagen, is a major constituent of basement membranes, where cells adhere and interact extensively with Type IV collagen. Collagen XV and XVIII, the other non-fibrillar collagens, are also expressed in the basement membrane. Although collagen fibers are generally a heterogeneous mixture of different types, within a given tissue one type of collagen usually predominates. The fibrillar collagens influence multiple aspects of cell behavior by serving as ligands for integrin and non-integrin receptors, and as a reservoir of growth factors and peptide mediators. By their physical characteristics such as, fiber size, organization, density, stiffness, and pore size between fibers, they influence cell behavior. Recently, several studies have demonstrated that cancer progression results in structural changes in the collagenous stroma [6-11].

Elastin, another major ECM fiber, provides recoil to tissues that undergo repeated stretch. Elastin stretch is limited by tight association with collagen fibrils [12]. A third fibrous protein, fibronectin is involved in directing the organization of the interstitial ECM and plays a crucial role in mediating cell
attachment and function [1]. Fibronectin is also important for cell migration during development and has been implicated in cardiovascular disease and tumor metastasis [5,13].

Proteoglycans, the other type of macromolecular constituent of the ECM, fill the majority of the extracellular interstitial space within the tissue in the form of a hydrated gel [2]. Proteoglycans are composed of glycosaminoglycan chains covalently linked to a protein core [3,14]. The glycosaminoglycan chains on the protein core are unbranched polysaccharide chains composed of repeating disaccharide units that can be divided further into sulfated (chondroitin sulfate, heparin sulfate and keratin sulfate) and non-sulfated (hyaluronic acid) glycosaminoglycans [3]. These molecules are extremely hydrophilic and adopt highly extended conformations that are essential for hydrogel formation and that enable matrices that are formed by these molecules to withstand high compressive forces. Proteoglycans have a wide variety of functions that reflect their unique buffering, hydration, binding and force-resistance properties. Many genetic diseases have been linked to mutations in genes encoding proteoglycans [2].

Function of stroma in health and pathology

Stroma surrounding the normal epithelial tissue is composed of non-activated adipocytes and fibroblasts which secrete and organize fibrous proteins such as type I and type III collagens, elastin, fibronectin, and proteoglycans such as hyaluronic acid. Tissue homeostasis is achieved by a balance between metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), controlled activity of crosslinking enzymes such as transglutaminases, and a number of ECM-bound growth factors that modulate cell growth and migration via a tightly controlled feedback circuit [1,15,16]. Aging is associated with a number of changes in the ECM. Thinning of the basement membrane occurs as a result of elevated MMP-mediated degradation and reduced protein synthesis. Elevated levels of interleukins and cytokines result in inflammation. The collagen fiber network is modified because of inappropriate collagen crosslinking which renders the tissue less elastic, more weak and rigid. This compromised ECM organization is believed to promote age-related diseases such as cancer [1,17-19]. ECM changes occur also as a response to acute injury which induces wound healing. Typically, this process involves the formation of a fibrin clot, stimulation of monocyte infiltration to the damaged ECM, and release of growth factors, MMPs and cytokines that promote angiogenesis and fibroblast migration and proliferation. The fibroblasts then synthesize and deposit large amounts of ECM proteins such as collagens, fibronectin
and hyaluronic acid. In healthy tissue, this ECM remodeling is controlled by feedback mechanisms and tissue homeostasis is restored.

Tumor stroma displays characteristics expected of unhealed wound. There is increasing evidence that ECM remodeling plays an active role in tumor progression and invasion. Inflammation and infiltrating T lymphocytes activate fibroblasts and induce their transdifferentiation into myofibroblasts [1] which deposit large quantities of ECM proteins and growth factors. Aberrant crosslinking of newly deposited collagen and elastin fibers generate more rigid fibrils that stiffen the tissue. MMPs secreted and activated by tumor cells and myofibroblasts also remodel the basement membrane surrounding the tumor and release ECM-embedded growth factors. Vascular permeability and new vessel growth are promoted by these growth factors which facilitate tumor invasion and metastasis.

**ECM in pancreatic cancer**

Pancreatic ductal adenocarcinoma is characterized by a fibrotic stroma with excessive connective tissue. The resistance of these tumors to all therapies has been traced to its pathological features [20]. Sparse vascularization coupled with copious deposition of extracellular components define the stroma in pancreatic cancer. The desmoplastic stroma surrounding the cancer cells participates in tumor-stroma interactions that promote pancreatic cancer cell invasion and metastasis [21]. The progression of precancerous PanIN lesions to pancreatic cancer is accompanied by changes in stroma. Early PanIN lesions may be associated with small amounts of normal stroma surrounding the normal pancreatic ducts. By contrast, PanIN 3 lesions begin to display enhanced stroma formation and invasive carcinoma is associated with extensive stroma. Although the cancer cells themselves are capable of synthesizing and releasing collagens, in PDAC, it is the stromal cells that are involved in ECM protein synthesis. Pancreatic stellate cells (PSCs) have emerged as important regulators of desmoplasia in pancreatic cancer [22] (see Chapter 3). The stroma in PDAC consists of proliferating fibroblasts and pancreatic stellate cells that produce and deposit fibronectin and collagens I and III [23]. Altered gene expression profile in the cancer-associated stroma might be responsible for the increased expression of collagen I.

Many epithelial malignancies such as breast, prostate, ovarian and pancreatic cancers exhibit a significant stromal reaction around tumor cells. Compared to other epithelial malignancies, pancreatic cancer displays the most prominent stromal reaction [24]; however, study of the epithelial-stromal interaction in pancreatic cancer has been initiated only recently. This
lack of investigation of stroma in pancreatic cancer may be due to limited access to tumor tissue which may be alleviated by the availability of suitable animal models (see Chapter 4). Results of the few animal studies that have investigated the role of ECM in pancreatic cancer appear to mimic the situation in human pancreatic cancer. Tumors produced by injection of a mixture of PSCs and cancer cells had increased fibrosis and larger tumors (see Chapter 3). In another study by the same group, coinjection of PSCs with cancer cells overexpressing the serine protease inhibitor SERPINE2 into nude mice was reported to result in increased tumor growth [22]. These findings suggest that the increased ECM (particularly fibrillar collagen) deposition as a consequence of protease inhibition might facilitate cancer progression. A mutant Kras (G12D) progression mouse model of pancreatic ductal adenocarcinoma generated extensive ductal lesions and the acinar parenchyma was replaced by an intense desmoplastic reaction composed of collagen, fibroblasts, and inflammatory cells similar to human pancreatic cancer [25] (as discussed in Chapter 4).

Histopathological analyses of human pancreatic ductal adenocarcinoma (see Chapter 1) depicted dense collagen I and III bundles associated with fibroblasts with loss of basement membrane integrity and invasion of malignant cells into the interstitial matrix with exposure to collagens [26]. An in vitro investigation found that pancreatic cancer cells that were grown on type I collagen had a higher proliferative capacity most likely due to the overexpression of antiapoptotic protein Mcl-1 [27]. Another immunohistochemical study of human pancreatic cancer that used digital image analysis to quantify the expression of ECM proteins in tumor stroma [28] reported that the overall surface covered by collagen types I and III and fibronectin was significantly higher in tumor stroma than in normal tissue. In contrast to normal pancreas, other ECM proteins such as laminins, collagen type IV, and vitronectin were partially lost in the basement membranes of pancreatic cancer. In addition, laminins, fibronectin, and collagen type IV were found to increase tumor cell migration. It has been suggested that key ECM proteins such as collagen and fibronectin likely interact with cell surface integrin receptors to provide survival signals to pancreatic stellate cells and pancreatic cancer cells [26].

The role of stroma in the drug resistance of pancreatic cancer (see Chapter 8) has been experimentally verified using a mouse model of the disease [29]. In the context of the physiological resistance to drug treatment that solid tumors possess, it has been postulated that anomalous assembly of the collagen network component and its interaction with the proteoglycans component of the tumor ECM could greatly influence the physiological barrier to macromolecule motion posed by healthy tissue ECM [30]. Using
in vitro experiments, it was verified that tumors with a well-defined collagen network are more resistant to penetration by macromolecular drugs compared with tumors that exhibit a loose collagen network. It was further determined that macromolecule access to tumor tissue is dominated by deficiencies in collagen assembly and is relatively insensitive to variations in glycosaminoglycan content. These results identify ECM characteristics of tumors, particularly pancreatic cancer, which could be useful in predicting penetration by therapeutic macromolecules. Imaging methods that are sensitive to the changing ECM characteristics of tumors are highly valuable as they are likely to have both diagnostic and predictive potential in assessing pancreatic cancer. In this context, it must be remembered that to date the microstructure of pancreas in its healthy state has not been explored in detail using any single imaging modality [31].

**Imaging the ECM**

The extracellular matrix is a complex network of glycoproteins and proteoglycans that originated with the advent of multicellular organisms [4]. However, most direct imaging of ECM in tumors has been focused on imaging collagen, the most abundant protein in mammals. This is in part due the significance of collagen network remodeling in desmoplastic reaction associated with tumors, and to a large extent due to the availability of optical imaging methods to visualize changes in fibrillar collagen. Atomic resolution techniques such as atomic force microscopy and X-ray diffraction technique have been used to determine molecular structures of collagen fibrils from tendon [32,33] but are not useful for in vivo applications. The combined use of multiphoton microscopy (MPM) and second harmonic generation (SHG) imaging have been useful in monitoring the interaction of cancer cells with the surrounding stroma [34]. MPM is a variation of conventional laser scanning confocal microscopy and has become the method of choice for the investigation of single cell dynamics in living tissue specimens and in vivo [35]. MPM allows intrinsic contrast imaging, in addition to fluorescence based contrast. The primary sources of intrinsic contrast in MPM are the fibrillar collagen and elastin, which are the primary ECM constituents, as well as certain metabolites such as NAD and NADH. Collagen fibers and other highly repetitive chiral structures interact with femtosecond light pulses causing nonlinear light scattering and interference termed second harmonic generation (SHG), which produces visible light of exactly half the wavelength of the illumination [36,37]. The structure of ECM can thus be revealed by simultaneous imaging of collagen and elastin autofluorescence. By using SHG and MPM, ECM was analyzed in esophageal cancer showing
that desmoplastic collagen fibers lost their typical fine structure [38]. In a breast cancer model, tumor-associated collagen signatures that could be used as characteristic markers of tumor expression were identified [39].

SHG was first demonstrated by Kleinman in crystalline quartz in 1962 [40]. Recent studies of the three-dimensional \textit{in vivo} structures of well-ordered protein assemblies such as collagen, microtubules, and muscle myosin are beginning to establish second harmonic imaging microscopy (SHIM) as a non-destructive imaging modality that holds promise for both basic research and clinical pathology. There are a number of detailed reviews on the theory, instrumentation and biological applications of SHG imaging and SHIM [36,41,42]. Therefore, we will discuss only the applications of SHG to image collagen in tumor tissues. SHG is a second-order nonlinear optical process that has symmetry constraints confining signal to regions lacking a center of symmetry. The basic instrumentation requirements for SHG microscopy are those for two photon fluorescence microscopy, namely, a scanning microscope coupled to a pulsed infrared laser [41,42]. When intense laser light passes through a highly polarizable material with a noncentrosymmetric molecular organization, the second harmonic light emerging from the material is at precisely half the wavelength of the light entering the material. This process, known as SHG, changes two near-infrared incident photons into one emerging visible photon at exactly twice the energy (and half the wavelength). Similar to two-photon absorption, the amplitude of SHG is proportional to the square of the incident light intensity. Therefore, SHG microscopy resembles two-photon microscopy in its intrinsic optical sectioning characteristics. As opposed to two-photon microscopy, SHG does not involve an excited state; as a result, energy is conserved and the coherence of laser light is preserved in SHG. Because of this coherence, most of the signal wave propagates with the laser. The exact ratio of the forward to backward signal is dependent upon the sample characteristics. SGH signals are directly obtainable from several structural protein arrays, including collagen, without the use of exogenous molecular probes. Biological materials can be highly polarizable and often assemble into large, ordered noncentrosymmetric structures. It is this property of collagen fibrils and their relevance in tumor microenvironment that we discussed earlier that has made collagen the target of several recent tumor studies.

In 1986, Freund et al. [43] used SHG microscopy to study the endogenous collagen structure in a rat tail tendon at \(~50\mu\text{m}\) resolution. Four types of collagen (I, II, III and V) form fibrils, type IV forms sheets in basement membrane and types VI and IX bind collagen to other cell components [42]. Type I collagen is highly crystalline and is an effective generator of second harmonics. SHG has the potential to distinguish between
different collagen types as well as the sensitivity to image collagen at high resolution, with diffraction-limited resolution near 300nm [44,45]. The relative alignment of fibrils and fibers is reflected in the magnitude of $\chi^2$, which is experimentally manifested in the SHG intensity [46]. The forward-backward ratio carries information related to the sub-resolution size and packing of the fibrils and fibers. SHG has the advantage over TEM in being able to image noninvasively through intact 3D tissues with relatively large field of view.

Several recent studies have used SHG and multiphoton microscopy to image collagen in tumors and better understand how changes in density and matrix organization are related to tumor formation and progression. In an ex vivo imaging study of epithelial-stromal interactions in normal mammary glands, mammary tumors and tumor explants, local alterations in collagen density was clearly seen around tumors [10,39]. In addition to detecting alterations in collagen density, changes in the three-dimensional organization of collagen fibers was imaged in tumors using multiphoton laser-scanning microscopy to generate multiphoton excitation of endogenous fluorophores and SHG to image stromal collagen. Based on the observed changes in collagen organization, Keely, et al. have defined three tumor-associated collagen signatures (TACS) that could provide novel markers to diagnose and characterize breast cancer.

More importantly the SHG-imaged TACS-3, defined as collagen fiber bundles oriented perpendicular to the tumor boundary, has been reported to predict the long-term survival rate of human patients [47].

Remodeling of the ECM has been implicated in ovarian cancer as well. Campagnola et al. [48] found that SHG emission attributes such as directionality and relative intensity which are related to the tissue structure were different in biopsies of human ovarian cancer, indicating changes in the collagen assembly. Based on their SHG observations they concluded that the malignant ovaries were characterized by denser collagen as well as higher regularity at both the fibril and fiber levels. Sahai et al. [49] further showed that invasive cancer cells used collagen fibers to facilitate migration in vivo, emphasizing the need to understand the role of collagen in tumor ECM remodeling and highlighting the value of imaging ECM changes using techniques such as SHG microscopy. Classical histological techniques cannot provide such specific information on ECM alterations in tumors. It has further been shown that SHG signal characteristics are sensitive to the type of collagen that is present and therefore, changes in ECM composition can be detected using this technique. In a detailed investigation using both model systems and mouse models to examine the capabilities of SHG for dynamic imaging of collagen modulation in tumors, Jain et al. [50] demonstrated SHG
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Figure 1. Tumor-associated collagen signatures. (a)-(c) Example of TACS-1. A region of locally dense collagen (a) near (40 μm 'above') a small tumor region (b) that is within the globally increased collagen region surrounding tumors, resulting from increased SHG (collagen) signal intensity; (c) three-dimensional surface plot of intensity showing an approximately three-fold signal increase at TACS-1. (d), (e) Example of TACS-2, showing straightened (taut) collagen fibers stretched around and constraining an expanded epithelial tumor volume. At regions of TACS-2, quantitative analysis [39] of fiber angles relative to the tumor boundary shows a distribution of fibers around 0° that correlates to non-invading regions of tumor cells. (f) Example of TACS-3, showing radially aligned collagen fibers, reorganized by tumor cells, at regions of tumor cell invasion. At regions of TACS-3, quantitative analysis [39] of fiber angles relative to the tumor boundary shows a distribution of fibers around 90° that correlates with local invasion of tumor cells. (Figure reproduced from [10]).

signal specifically came from fibrillar collagen I and there was no signal from collagen IV which forms the basement membranes. Up regulation of collagen V has been implicated in human breast cancer. Using the metrics of SHG intensity, fiber length, emission directionality and depth-dependent intensities, quantitative discrimination of type I and type V collagens was possible in self-assembled collagen gels that were model systems for ECM in invasive breast cancer [46]. To date there has been no SHG study of ECM
changes in pancreatic cancer either in animal models or on human tissue although ELISA measurements have indicated changes in type IV collagen in pancreatic cancer. In vitro pancreatic cancer cells were found to produce and secrete more of type IV collagen [51]. The Swedish group also found that patients with pancreatic cancer had increased circulating levels of type IV collagen. Since collagen IV does not have a SHG signal, it is yet unclear how these promising findings could be exploited by nonlinear imaging techniques. Structural differences between type I and type IV collagen in a number of biological tissues, including lens capsule, sclera, and tendon, have been investigated in vivo by another spectroscopic method called attenuated total reflection/Fourier transform infrared spectroscopy [52].

Both direct and indirect approaches have been employed to examine the pancreatic ECM using MR imaging. Time-signal intensity curve (TIC) obtained from dynamic magnetic resonance imaging was found to be useful in the evaluation of pancreatic fibrosis after pancreaticojejunostomy in patients undergoing pancreaticoduodenectomy [53]. A time-intensity curve (TIC) with a rapid rise to a peak followed by a rapid decline was characteristic of a normal pancreas without fibrosis. Pancreatic TICs with a slow rise to a peak followed by a slow decline or a plateau indicated a fibrotic pancreas. A study that prospectively analyzed dynamic contrast enhanced (DCE) MRI data from patients with focal pancreatic lesions found that histologically measured pancreatic fibrosis was negatively correlated with the rate of contrast agent uptake, and positively correlated with tissue volume fraction occupied by extravascular extracellular space [54]. In this study, rate constant of contrast agent uptake and extravascular extracellular space were calculated from DCE MR parameters, and fibrosis was semiquantitatively estimated by picrosirius staining. While these indirect imaging approaches have shown potential for evaluating pancreatic fibrosis, they are confounded by changes in vasculature associated with pathology, which was clearly illustrated in a contrast enhanced CT study [55].

Direct visualization of the mouse pancreas architecture has been reported using MR microscopic imaging [56]. In this approach, high spatial resolution images were acquired ex vivo from normal mouse pancreas without contrast agents on a high field strength imager. The resulting images were analyzed using volume rendering to resolve components in the pancreas such as islets, acinar cells, blood vessels and ECM. The three-dimensional architecture of the extracellular matrix which appeared as sheets could be characterized using MR microscopy. Using MR microscopy, extensive fibrosis was observed in older Pdx-Cre/LSL-Kras mouse pancreas, which displayed neoplastic lesions (Venkatasubramanian PN, et al. Unpublished results).
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Figure 2. MR microimage of normal mouse pancreas. A. 2D view of a representative slice from a three-dimensionally acquired MR image. B-D. 3D volume-rendered images of the boxed area in A. The color scale corresponds to the magnetic resonance signal intensity (green to red, low to high intensity). Cylindrical tube-like and planar sheet-like features can be identified in the high-intensity region of the volume image, in addition to islets, which appear ellipsoid. Note that these tubes and sheet-like features, both of which appear as high-intensity regions, cannot be clearly distinguished in the 2D view. B. A sheet (blue and cyan arrows) is seen underneath islets (red arrows). A tube appears in the bottom right corner (gold arrow). The sheet was measured to be ~116 μm thick along the y axis (cyan arrows) and ~298 μm in depth along the z axis (blue arrows). The total depth of this sheet through the tissue along the z axis (not shown) is ~1400 μm. C. 3D image of the tube (gold arrow) shown in B in a different location with surrounding islets (red arrows). The diameter of the tube is ~88 μm. The total length of the tube when tracked through the tissue (not shown) was measured to be ~5850 μm. D. 3D image of a tube with a high intensity lumen (red) and the lower intensity wall (green). Note the clear distinction in morphology between the tube and the planar sheet. (Figure reproduced from [56]).

Another potential target for ECM imaging in abnormal stromal extracellular matrix remodeling can be hyaluronan metabolism. Hyaluronan in the ECM provides a favorable microenvironment for cell proliferation and migration, in addition to activating intracellular signals through interaction with cell surface receptors. Progression of breast cancer and many other cancers have been associated with elevated hyaluronan metabolism [57,58]. High levels of hyaluronan within tumor cells or in the peritumor stroma have been observed in many cancers and have been suggested to be prognostic indicators of poor outcome in breast, ovarian, gastric and colorectal cancers [59-61]. Changes in ECM hyaluronan levels are known to occur in pancreatitis [62] and pancreatic cancer [63]. Recently, many molecular...
imaging probes have been developed to report hyaluronan metabolic activity [64-69]. It has been suggested that visualization of the ECM in the MR microscopic images of normal mouse pancreas was based on the strong water-binding capacity of hyaluronan, a glycosaminoglycan [56]. Based on this study, MR microscopy can be a viable technique to indirectly image the ECM via changes in water content associated with hyaluronan levels in pancreatitis and pancreatic cancer.

**Summary and conclusions**

ECM is a non-cellular component of tissues that provide physical scaffolding and participate in physiological function by interacting with cell-specific receptors to transduce various signals within cells. The properties of ECM vary from tissue to tissue and from one physiological state to another. ECM, which is composed of proteins including collagen, polysaccharides such as proteoglycans, and water is a dynamic structure that is being constantly remodeled, especially in cancer. Many epithelial malignancies including pancreatic cancer exhibit prominent stromal reaction that involves alterations in ECM collagen characteristics. Non-linear optical imaging methods have been used to image this ECM remodeling in breast, ovarian and gastric cancers. Second Harmonic Generation or SHG imaging can directly detect signal from type I collagen which is the predominant component of ECM in healthy and cancerous tissues. SHG has been used to measure alterations in ECM composition such as increased levels of collagen in tumor stroma. Reorganization of type I collagen fibrils and fibers occurs in the tumor stroma in breast, ovarian and other epithelial cancers and SHG signal characteristics are sensitive to such architectural changes. Increased stromal reaction has been known in pancreatic cancer; however, it has not been examined using SHG. It has been established that pancreatic cancer is associated with increased levels of type IV collagen; but SHG cannot image this nonfibrillar collagen. The levels of hyaluronan, a proteoglycan of the ECM, has been known to change in pancreatitis and pancreatic cancer. Molecular imaging using probes specific for hyaluronan metabolism can thus be used to image ECM changes in pancreatic diseases. Another technique that showed potential in imaging pancreatic ECM without the use of exogenous contrast agents is MR microscopy using high field strength imagers. Whereas SHG is limited in depth and field of view, MR microscopy has the ability to image the entire organ. While these molecular, optical and magnetic resonance imaging approaches have the potential for visualizing the ECM changes that are associated with pancreatic diseases such as pancreatic cancer, the promise these techniques hold has not been exploited. A better
understanding first of the specific ECM alterations at the molecular level would perhaps provide the knowledge needed to construct specific imaging tools for better diagnosis of pancreatic diseases.

References

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