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Review Article

Special cancer microenvironment in human colonic cancer: Concept of cancer microenvironment formed by peritoneal invasion (CMPI) and implication of subperitoneal fibroblast in cancer progression

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Clinical outcomes of colorectal cancer are influenced not by tumor size, but by spread into the bowel wall. Although assessment of serosal involvement is an important pathological feature for classification of colon cancer, its diagnostic consistency has been questioned. Using elastic staining, we assessed elastic laminal invasion (ELI) for more objective stratification of deep tumor invasion around the peritoneal surface. In addition, pathological characteristic features of marked tumor budding, fibrosis, and macrophage infiltration in the tumor area with ELI was elucidated. This characteristic tumor area was termed cancer microenvironment formed by peritoneal elastic laminal invasion (CMPI). We elucidated histoanatomical layer-dependent heterogeneity of fibroblast in colonic tissue. Furthermore, subperitoneal fibroblasts (SPFs) play a crucial role in tumor progression and metastasis in CMPI. Our ELI and CMPI concept contributes not only to objective pathological diagnosis, but also sheds light on biological research of special cancer microenvironments detectable in human colorectal cancers. Herein, we describe the diagnostic utility of ELI and morphological alteration in advanced colorectal cancers to determine the phenomenon that occurs when tumors invade around the peritoneal surface. Next, biological research of CMPI is reviewed to stress the importance of pathological research to establish new biological concepts.

Key words: cancer associated fibroblast, cancer microenvironment, colorectal cancer, elastic laminal invasion, fibroblast

The prognosis of gastrointestinal cancers is not influenced by the tumor size, but is instead strongly influenced by the tumor spread. Since the first categorization effort by Lockhart-Mummery, primary colorectal cancer has been consistently stratified based on the extent of spread into the bowel wall.[1,2] Deep tumor invasion around the peritoneal surface has also been reported as a prognostic factor (invasion through all the layers, peritoneal involvement, or direct spreading involving a free serosal surface), and the current pT4a definition of 'tumor perforates visceral peritoneum' was established.[3–5] Although many reports have supported this definition of pT4a as a prognostic factor, difficulty of its assessment has also been reported.[6] The first aim of our research was the objective assessment of tumor invasion around the peritoneal surface using elastica stain.[7] We reported that peritoneal elastic laminal invasion (ELI) was an independent risk factor for colon cancer, which suggested diagnostic utility in daily practice. Furthermore, detailed morphological analysis revealed characteristic histologic findings of marked fibrosis, tumor budding, and macrophages in the tumor area with ELI.[2] We defined this pathologically detectable special tumor area created between the peritoneal elastic lamina and peritoneal surface as a cancer microenvironment formed by peritoneal invasion (CMPI).[8] Definition of ELI and CMPI enabled further pathological and biological research. At first, using the morphological pattern of ELI and CMPI, we were able to estimate morphological alterations in advanced colorectal cancers. Corresponding clinical features of bowel obstruction

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through this morphological alteration were also elucidated.[9] Next, we identified subperitoneal fibroblasts (SPFs) and their activation as crucial factors that enhance tumor progression and metastasis in CMPI. Furthermore, using global gene expression analysis, we elucidated orchestrated regulation of SPFs activation, M2 macrophage, and EMT associated gene expression in human colon cancer tissue. This pattern was similar to morphological features of CMPI (prominent tumor budding, fibrosis, and macrophages), and consistent with the concept of the cancer microenvironment.[10] Successful biomarker research using the gene expression profile of SPFs activated by cancer conditioned medium has also been performed. In this review, first, we summarize the diagnostic utility of ELI and speculate on the morphogenetic features of advanced colorectal cancers. Next, biological and biomarker studies based on ELI and the CMPI concept are also reviewed to determine the importance of pathological research to establish new biological concepts.

Assessment of tumor spread and ELI in colon cancer

The basic function of the large bowel is conservation of salt and water and facilitation of orderly disposal of waste materials.[11] The gastrointestinal tract consists of five histoanatomical layers, which are the mucosal, submucosal, muscular, subserosal, and serosal layers. These layers consist of a variety of cell types, and although the layers have distinct roles, they orchestrate their activities to accomplish basic functions. Gastrointestinal cancers originate from the surface structure of the mucosal layer, and then invade into deeper layers of the wall in a fixed order. As mentioned earlier, the clinical outcome of gastrointestinal cancers is known to be strongly influenced by this tumor spread. However, genetic alterations responsible for tumor spread have not been identified. Heterogeneity of these histological layers may produce a heterogeneous reaction to the cancer stimulation, thereby creating a heterogeneous cancer microenvironment, and contribute to the tumor-spread-dependent clinical outcome. Many early reports of the classification of tumor spread focused mainly on rectal cancer. The first classification by Lochart-Mummery was as follows: (A) favorable cases, tumor did not invade the muscularis; no nodes involved; (B) medium cases, tumor invaded muscular coat; no extensive involvement of nodes; and (C) very bad cases, tumor large and fixed; or extensive involvement of nodes. The outline for the current pT1-3 stages in TNM classification was already established by 1949, and this classification has been known to be applied also for the assessment of colon cancer.[1,12,13] The current definition of pT1-3 is as follows: pT1, tumor invades submucosa; pT2, tumor invades muscularis propria; pT3, tumor invades subserosa or into non-peritonealized pericolic or perirectal tissue. The next challenge was to classify colorectal cancer spreading beyond the bowel wall. Free mesothelial surface involvement or local peritoneal involvement, which is relevant to the current pT4a, was reported to be a prognostic factor.[3,5] They challenged to sub-stage tumors spreading beyond the bowel wall using the landmark of the peritoneal surface. The current definition of pT4a is tumor perforating the visceral peritoneum, and many reports support this definition as a prognostic marker. However, difficulty in the accurate determination of pT4a was also reported. The peritoneal surface is not smooth, but instead exhibits peritoneal clefts or reflections where serosal involvement is frequently seen. In addition, subperitoneal fibro-inflammatory changes often disturb the clear determination of pT4a (Fig. 1).[6] A Japanese survey revealed that the frequencies of pT4 cases in resected colorectal cancers were 11.2%–67.7%.[14] This marked variability seemed to be associated with the difficulty in the diagnosis of pT4a. The peritoneal elastic lamina, which is situated immediately beneath the basement membrane of mesothelial cells, can be another landmark for sub-stage tumor spreading beyond the bowel wall, and we performed a clinicopathological study of ELI. Topographical investigation of human peritoneal elastic lamina was performed by Knudsen *et al*. The peritoneal elastic lamina has been known to exist ubiquitously in visceral and parietal peritoneum with quantitative variety depending on the properties of the organ it covers. In the normal intestines, although not a complete circle, prominent peritoneal elastic lamina has been known to be found and can be followed all the way to the posterior abdominal wall.[15] We determined that, in addition to results showing that ELI is an independent prognostic factor, the prognosis of patients with pT4a stage II colon cancer was equivalent to that of patients with ELI-positive stage II. Therefore, the prognosis of colon cancer was thought to not be influenced by the perforation of the visceral peritoneum, but instead by ELI.[7] The malignant potential of colon cancer was thought to be suddenly up-regulated just when it invades beyond this very thin peritoneal elastic lamina. A similar phenomenon was also reported in lung cancer, and the interaction of cancer cells and serosal tissue seemed to be an intriguing context in cancer biology.[16]

Morphological features and morphogenetic analysis of the cancer microenvironment formed by ELI

Why is clinical outcome influenced by ELI, and which cell components are associated with this phenomenon? Recently, tumors have been recognized as one organ which consists of various mesenchymal and inflammatory cells. And this concept is known as the cancer microenvironment.[17,18] The cancer microenvironment was thought to be heterogeneous within a single tumor. Concordant morphology with a heterogeneous microenvironment is often observed in the invasive front of colorectal cancer. For

example, desmoplastic reaction or tumor budding, without tubular structures, are known to be predominant features in the invasive front of colorectal cancer.[19] In colon cancer, the tumor area with ELI is located in the invasive front of the tumor (Fig. 2d, e). However, the morphological features of the tumor area with ELI have not been elucidated. Our observation of tumors with ELI revealed that ELI is often accompanied by peritoneal indentation (Fig. 2a–c). Further detailed morphological study revealed characteristic pathological features of prominent budding foci, fibrosis, and macrophages (Fig. 3).[2,7,20] This pathologically detectable tumor area with ELI was defined as a cancer microenvironment formed by elastic laminal invasion (CMPI). Tumor budding and macrophages are more prominent in the periphery of CMPI, around the boundary of the elastic lamina. In the center of CMPI, fibrosis and abundant ECM components were observed. Fibroblasts in CMPI showed prominent α-smooth muscle actin that suggested marked activation (Fig. 3h). Histologically, ELI can be classified into elevated and non-elevated type (Figs 3b,f, 4a).[9] In cases of elevated type ELI, the peritoneal elastic lamina is drawn towards the tumor, and more marked macroscopic indentation of the peritoneal surface can be observed. Cases with elevated type ELI show a larger CMPI area, more prominent lateral tumor spread, and higher tumor annularity rate than do the non-elevated type (Fig. 4a). These results seemed to suggest the progression from non-elevated type to elevated type. In addition, bowel obstruction was more frequently seen in cases with elevated type ELI. However, the clinical outcome was not different between them. Therefore, prognosis was influenced by the formation of CMPI, and clinical symptoms of bowel obstruction appeared after the growth of CMPI with a delay. Morphologically, CMPI in colon cancer was thought to spread into the tumor surface and alter whole tumor stiffness. The interaction of cancer cells and peritoneal tissue beyond the peritoneal elastic lamina seemed to cause drastic changes detectable histologically and macroscopically. Histologically, the area between the peritoneal elastic lamina and the surface consisted of subperitoneal fibroblasts (SPF) and extracellular matrix.[11] Therefore, we investigated the interaction between cancer cells and SPFs, and its contribution to cancer progression and metastasis.

Subperitoneal fibroblasts in CMPI contribute to tumor progression and metastasis

History and histology of fibroblasts, myofibroblasts, and cancer associated fibroblasts

The existence of fibroblasts is morphologically described in the 19th century.[21] Owing to their ability to adhere to plastic, cultivation of fibroblasts was also established in the early 20th century.[22,23] They distribute throughout the connective tissue of the whole body, and constitute a major cell component of the stroma.[24] In their development stage, fibroblasts originate from the mesoderm, and play a pivotal role in the formation of many organs.[25] In their adult stage, in addition to their basic function of contributing to the maintenance of a structural framework, they produce growth factors, cytokines, and extracellular matrix to maintain

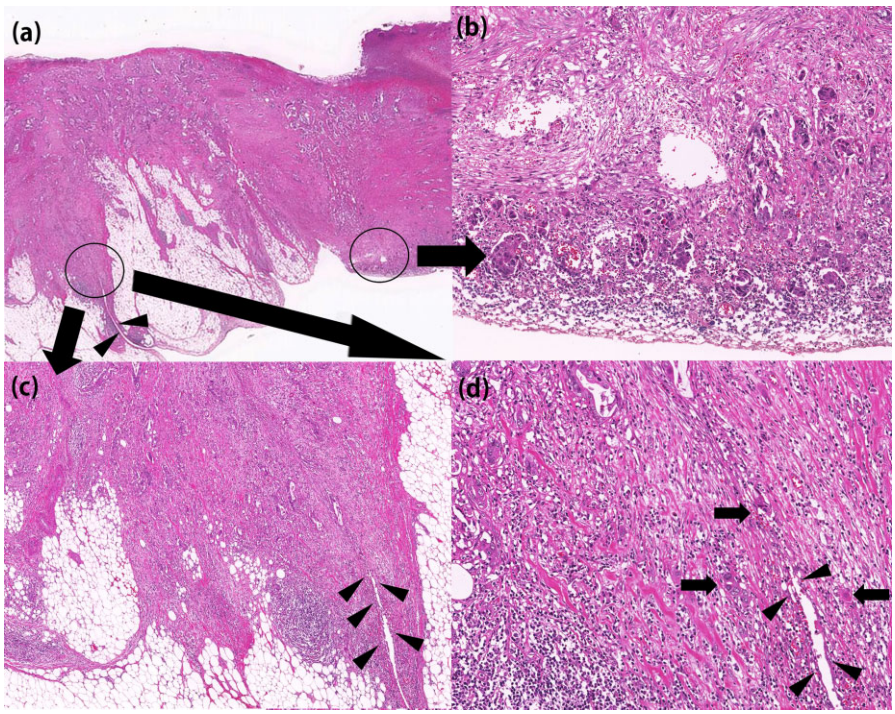

Figure 1 Difficulty in the determination of pT4a. (**a,c,d**) Peritoneal surface is not smooth, but exhibits peritoneal clefts or peritoneal reflection (arrow heads) where serosal involvement is frequently seen. (**b**) Subperitoneal fibro-inflammatory changes often disturb the clear determination of pT4a. Such lesions seem to cause institutional variability in tumor classification. (**d**) Cancer cells clustered near the peritoneal clefts are shown in arrows.

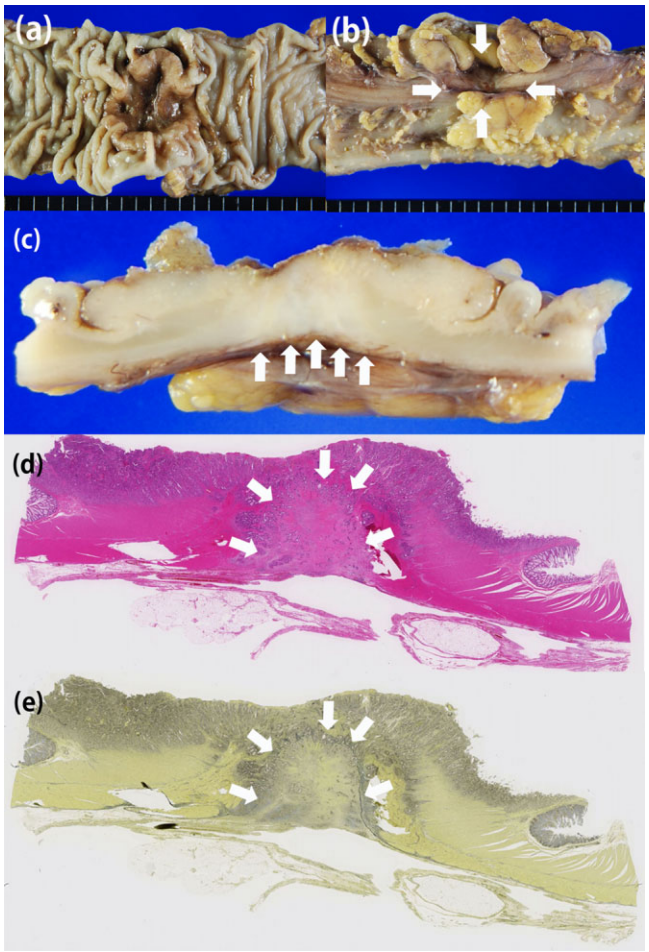

Figure 2 Macroscopic and loupe view of colon cancer with peritoneal elastic laminal invasion (ELI). (**a**) Macroscopic features of colon cancer from the luminal side. (**b**) Macroscopic features of colon cancer from the peritoneal side. (**b,c**) Note the indentation (arrows) in the cancer microenvironment formed by peritoneal invasion (CMPI). (**d,e**) Loupe view of hematoxylin and eosin (H&E) (**d**) and elastica stain (**e**). Tumor area under arrows is CMPI. CMPI can be identified clearly using H&E and elastica stain.

tissue homeostasis.[26] Histologically, fibroblasts are cells with long, flat, and spindle-shaped morphologic features that reside in connective tissue throughout the body. Their elongated cell body connects to a few spear-shaped processes.[24] The cytoplasm of the cells is also narrow and slightly eosinophilic. The large oval nucleus has delicate chromatin and one or more large nucleoli. In electron microscopy, well-developed Golgi bodies and rough endoplasmic reticulum are prominent (Fig. 5).[27] Though fibroblast-specific-protein 1 (FSP1) seemed to be the most specific, an unambiguous marker of fibroblasts is still lacking, which complicates biological investigation.[28] Fibroblasts in a pathological state show different morphology. Such a fibroblast is originally found in granulation tissue.[29] The name of 'myofibroblast' was proposed, based on the ultrastructural features resembling both fibroblast and smooth muscle cells, and the biological character of contractile ability.[30] Compared with normal fibroblasts, the cell body of the myofibroblast is more plump and possesses eosinophilic fibrillar cytoplasm with cable-like condensations.[11] Nuclei of myofibroblasts are more irregular than those of normal fibroblasts, and are often indented with granular chromatin and conspicuous nucleoli. Electron microscopic features of myofibroblasts share morphology with fibroblasts and smooth muscle cells. As with fibroblasts, Golgi bodies and rough endoplasmic reticulum are found. Also, similar to findings for smooth muscle cells, prominent cytoplasmic bundles of microfilament stress fibers run parallel to the long axis of the cells. Fibroblast activation protein-α, α-smooth muscle actin, or platelet-derived growth factor-β are representative markers for myofibroblasts.[28] Together with these marker expressions, myofibroblastic changes of fibroblasts by any stimulation is called activation. Such activated fibroblasts were prominent in the cancer microenvironment. Fibroblasts in cancer tissue or activated fibroblasts in cancer tissue are called cancer-associated fibroblasts (CAFs). Many CAFs keep their activated state through a few possible mechanisms, and accelerate cancer progression, epithelial mesenchymal transition, and metastasis.[31] The origin of myofibroblasts in the cancer microenvironment has been thought to be from residual fibroblasts in the adjacent tumor area.[18] Fibroblasts in the adjacent tumor area are influenced by cancer stimuli and many of them are activated. In addition to the heterogeneous activation status in cancer tissue, morphological and biochemical features of myofibroblasts that resemble normal fibroblasts support this thesis. In addition, local smooth muscle cells, endothelial cells or pericytes can be a source of myofibroblasts in cancer tissue.[32] Recently, bone marrow-derived progenitor cells are also reported as one candidate source of fibroblasts in cancer tissue.[33] Some reports even suggested the possibility of an epithelial origin of fibroblasts in cancer tissue. Indeed, we speculate that various cell types from many organs can be a source of fibroblasts in cancer tissue. However, pathologically, the amount of myofibroblasts is variable among different tumor origins. Breast cancer or gastric cancer is a representative tumor with abundant myofibroblasts. Traditionally, such a tumor has been called a scirrhous carcinoma. As a contrasting situation, scirrhous carcinoma in the liver or kidney is very rare. Recently, topological or organ dependent diversity of fibroblasts has been reported.[34,35] If all CAFs are derived from bone marrow, such a variety of tumor fibrosis may not be observed. Therefore, we believed that the heterogeneity of residual fibroblasts constitutes, at least, one factor that produces such an origin-dependent variety of tumor stroma.

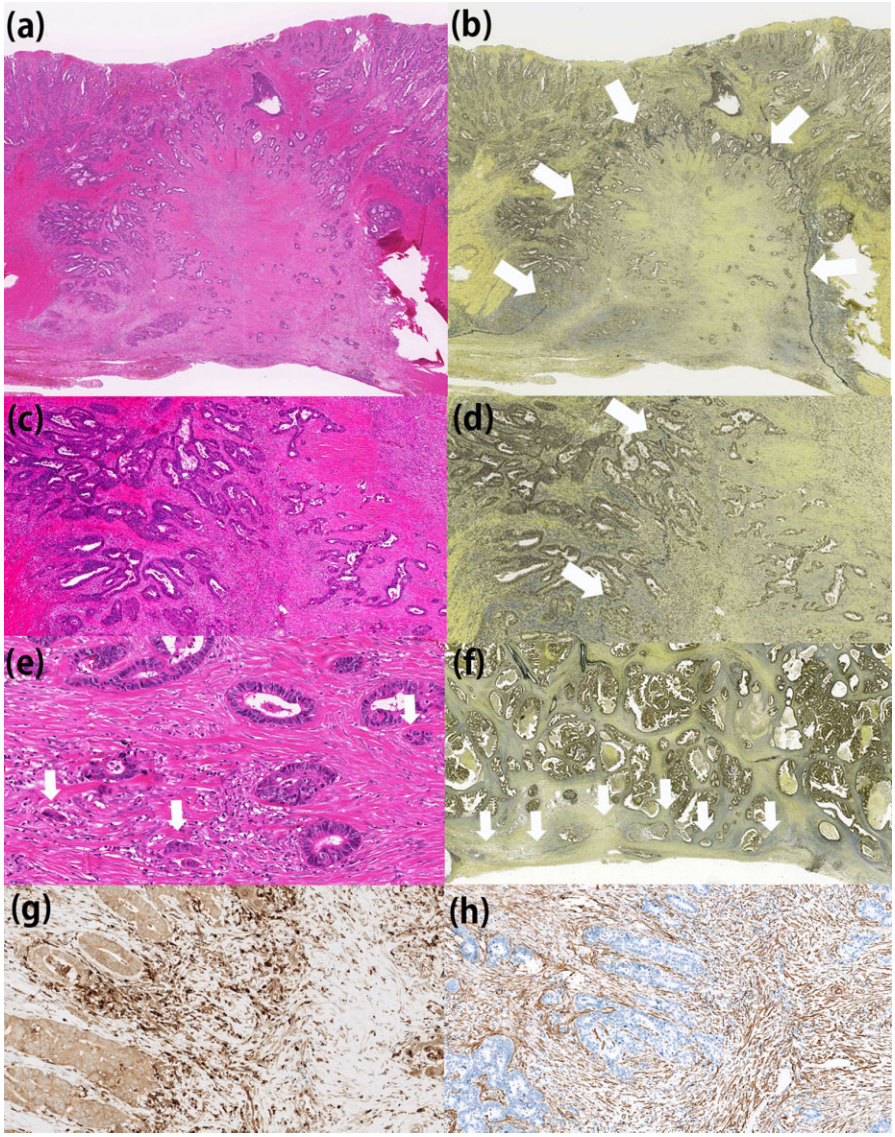

Figure 3 Histological features of elastic laminal invasion (ELI) and cancer microenvironment formed by peritoneal invasion (CMPI). (**a-d**) Histological features of the elevation type of ELI. Peritoneal elastic lamina is indented toward the tumor. (**a**) Low power view with H&E stain. (**b**) Low power view with elastica stain. CMPI is clearly identified below the arrow. (**c**) Peripheral area of CMPI with H&E stain. (**d**) Peripheral area of CMPI with elastic stain. Left side is not CMPI and right side is CMPI. Tumor histologic features are quite altered when tumors invade beyond the peritoneal elastic lamina. (**e**) Histologic features of CMPI. CMPI is abundant with activated fibroblasts. Prominent tumor budding is also seen (white arrow). (**f**) Non-elevation type of ELI. Case with non-elevation type of ELI showed similar prognosis with elevation type of ELI. On the other hand, they demonstrate smaller tumor size and less bowel obstruction than elevation type. Therefore, colon cancer seemed to progress from non-elevation type to elevation type of ELI. (**g**) CD68 expression in CMPI showed marked macrophage infiltration. (**h**) α-SMA expression in CMPI showed marked activation of fibroblasts.

Role of subperitoneal fibroblasts in colorectal cancer progression

Histologically, the area between the peritoneal surface and the peritoneal elastic lamina is less than 100 μm thick. Colorectal cancer invasion into this narrow space seemed to cause drastic histological and biological alteration. Rather than genetic alteration, CMPI induced by ELI was thought to be a tumor-promoting special microenvironment and to contribute to this phenomenon. Subperitoneal tissue consists of ECM components and SPFs. SPFs are known to generate large quantities of chemokines that can result in the failure of peritoneal dialysis.[36] The robust contractile ability of SPFs have also been reported to contribute to bowel obstruction in Crohn's disease.[37] Therefore, we formed a hypothesis that the reciprocal interaction between cancer cells and SPFs is one of the triggers to create CMPI and promote tumor progression and metastasis. At first, we propagated SPFs and SMFs, and compared biological features between SPFs and SMFs in a normal state. Although SPFs and SMFs shared spindle cell morphology, SPFs show less proliferation ability than do SMFs. In the global gene expression analysis, SPFs and SMFs formed distinct clusters. These results indicated a histoanatomical-layer dependent heterogeneity of fibroblasts in the normal colonic wall. SPFs showed more prominent expression of ECM associated genes than did SMFs, which is accordant with histology of the ECM-rich subperitoneal layer. Next, we evaluated the activity of fibroblasts by cancer conditioned medium stimulation. Surprisingly, SPFs showed more variable gene up-regulation than SMFs. SPFs specific up-regulated genes were enriched by actin-binding or contractile associated genes, including α-SMA encoding ACTA2, which is known as an activation marker and highly expressed in CMPI. Furthermore, in a comparison with SMFs, SPFs

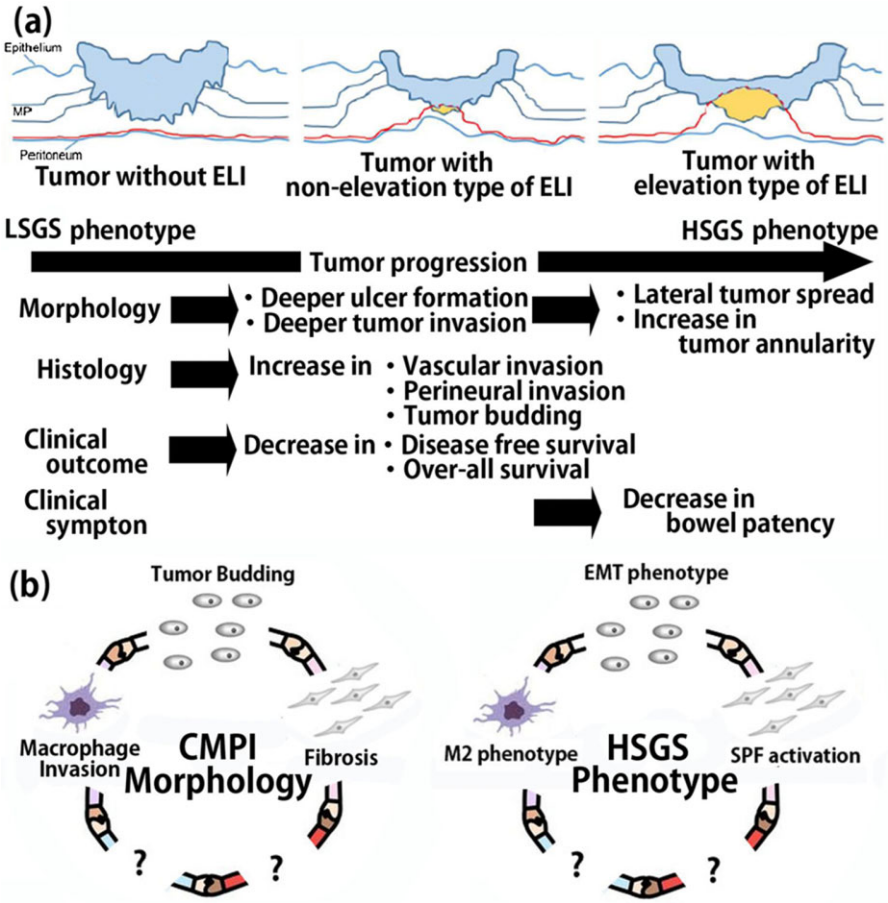

Figure 4 (**a**) Schema of estimated morphogenesis of advanced colon cancer based on elastic laminal invasion (ELI) and cancer microenvironment formed by peritoneal invasion (CMPI) concepts (partly revised from Ref9). Peritoneal surface is shown in blue line. Peritoneal elastic lamina is shown in red line. And CMPI is shown in yellow area. After progression into the subserosa, colon cancer invades beyond the peritoneal elastic lamina to become a tumor with non-elevated type of ELI. In this phase, tumor invasion and ulcer become deeper. Histologic features associated with prognosis including vascular invasion, perineural invasion, and tumor budding become more prominent; clinical outcome is drastically worsened. Together with the biological data, subperitoneal fibroblasts (SPFs) play a pivotal role in this phenomenon. After tumor invasion beyond the peritoneal elastic lamina, the elastic lamina is indented toward the tumor, and CMPI becomes larger to become a tumor with elevation type of ELI. In this phase, tumors become larger and tumor annularity is increased. Bowel patency is also decreased; SPFs activation is associated with decreasing bowel patency. However, the prognosis is not altered. The phenotype of advanced colon cancer is clearly classified by the expression of SPFs cancer cell conditioned medium response genes (SCR genes) in the high SPFs gene signature group (HSGS group) and the low SPFs gene signature group (LSGS) group. This seemed to reflect SPFs activation by ELI. (**b**) Common morphological and phenotypical features between CMPI morphology and HSGS phenotype. In CMPI, marked fibrosis, macrophage invasion, and tumor budding are characteristic features. SPFs activation is one of the triggers to create tumor-promoting CMPI. The HSGS group classified by SCR genes of fibroblasts also showed marked EMT-associated or M2 macrophage-associated genes. Therefore, these phenomena of fibrosis, macrophage invasion, and tumor budding were thought to be orchestrally regulated in human colonic cancer tissue.

were found to show more prominent contractile ability and α-SMA protein expression by stimulation with cancer conditioned medium.[9] Therefore, the prominent SPFs activation that occurred in CMPI was thought to induce bowel obstruction through the activation of contractile associated genes.

Next, cancer cells were co-injected with SPFs or SMFs into the SCID mouse. Tumors co-injected with SPFs resulted in more rapid tumor growth, enhanced tumor formation ability, and more frequent metastasis than that seen in the mice co-injected with SMFs. We concluded, based on these data, that histologically detectable CMPI in human colonic cancer was a cancer-promoting tumor microenvironment, and that in CMPI, SPFs play a crucial role in tumor progression and metastasis.

In addition to the previous report of organ-dependent and topological diversity, our results revealed histoanatomical-layer dependent diversity in colonic fibroblasts.[34,35] Special fibroblasts have been reported in the gastrointestinal tract. For example, pericryptal fibroblasts have been known to contact with the epithelial basement membrane, interact with epithelial cells, and contribute to the maintenance of mucosal homeostasis.[38–40] Fibroblastic cells of Cajal also serve as

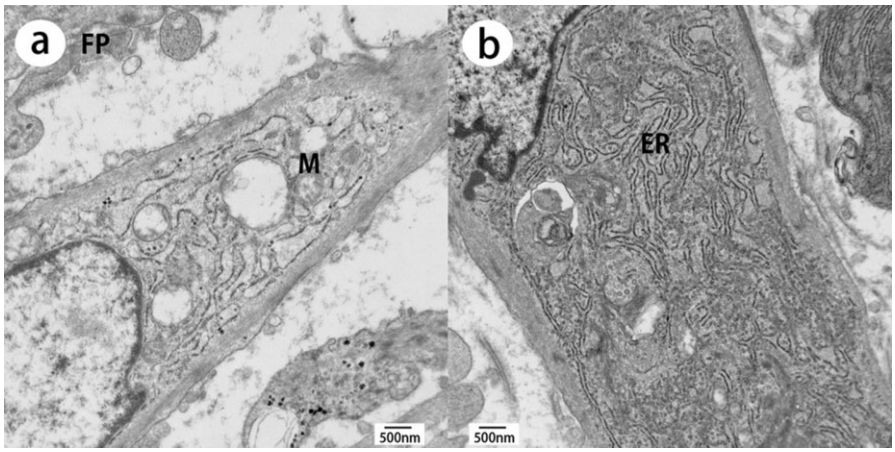

Figure 5 (**a,b**) Electron microscopic features of subperitoneal fibroblasts (SPFs). SPFs have long slender cell bodies with fusiform nuclei. Rough endoplasmic reticulum and Golgi complexes are prominent. Scattered mitochondria are also seen.

pacemakers of smooth muscle cells.[39] Therefore, the existence of a phenotypical and biological difference between SPFs and SMFs is not surprising. Rather the diversity of fibroblasts was thought to be supporting layer-specific function within the bowel wall. Further, heterogeneous fibroblasts were thought to constitute the heterogeneous reaction to cancer stimuli, and some of them may contribute to the formation of the special cancer microenvironment. Organ-dependent varieties of CAFs among different cancer models were reported by Earz *et al*.[41] Our results revealed that such a variety exists even within one organ. This diversity of fibroblasts creates a heterogeneous cancer microenvironment, and CMPI is a distinct cancer microenvironment detectable in human colonic cancer.[17] Our results also suggested that reactivity of residual fibroblasts is variable, and fibroblasts with high reactivity to cancer stimuli may more strongly promote tumor progression and metastasis. The association between reactivity and cancer-promoting potential should be more clearly elucidated.

Research using global gene expression data and biomarker research

Stromal features in wound healing and cancer progression have been known to share similar biological and pathological features.[42] Enhanced inflammation, blood coagulation ability, contractile ability, myofibroblast accumulation, and new vasculature induction have been reported to be common features in these pathological states.[43] Many of these features can be confirmed pathologically. Recent reports have revealed that *in vitro* microarray data can be applied to publicly released microarray datasets. Chang *et al*. showed that prognosis of many cancers was stratified by the microarray data of serum response genes in fibroblasts that represent wound response.[42] Colorectal cancer prognosis may also be stratified by the activated SPFs genes by cancer conditioned medium stimulation. Therefore, we tried to stratify publicly available microarray data of patients with colon cancer by using expression of SPFs cancer cell conditioned medium response genes (SCR genes).[10] Interestingly, a biphasic pattern of expression of SCR genes was observed in 226 colorectal cancers from GSE14333, and was divided into a high SPFs gene signature group (HSGS group) and a low SPFs gene signature group (LSGS group). Surprisingly, EMT associated genes of *ZEB1*, *ZEB2*, *TWIST1*, and *TGFB1*, and M2 macrophage associated genes of *CD163* and *MSR1* were expressed higher in the HSGS group than that seen in the LSGS group. These data suggested that SCR genes, EMT associated genes, and M2 macrophage associated genes were orchestrally regulated in human colon cancer tissue. Pathological tumor budding was thought to be a similar phenomenon with EMT.[44] This trait was similar to the pathological features of CMPI, which shows prominent fibrosis, tumor budding, and macrophages. Because ELI positive cases and the HSGS group showed poor prognosis, this trait was thought to be associated with tumor progression or metastasis. Genes highly expressed in the HSGS group or SCR genes were thought to be enriched in prognostic biomarkers. We constructed frameworks to select candidate markers, and after the validation, new prognostic biomarkers of *CALD1*, *TAGLN*, and *SPTBN1* were identified.[10]

Future perspective

Further observation of the histologic features in CMPI may reveal other cell components that play a role in tumor progression. A concordance study in the diagnosis of ELI will be available to determine how to objectively discriminate high-risk stage II patients. Objective discrimination of high-risk stage II cases will contribute to selecting cases available for postoperative adjuvant therapy.[45] Next, immunohistochemical study and functional analysis of newly identified

biomarkers will reveal biological mechanisms of cancer promotion by CMPI formation. Such studies may reveal new biological and molecular targets for cancer therapy. Recently, the mechanical features of the stroma have been reported to be associated with cell migration or development.[46] We identified that tumor-promoting SPFs have prominent contractile ability and induce α-SMA expression against cancer conditioned medium stimulation. α-SMA expression is known to be associated with tissue elasticity. Therefore, mechanical alteration of cancer tissue can be induced by SPF activation, and may also be associated with tumor metastasis. In fact, cases with ELI were known to show higher tumor elasticity as measured by a tactile sensor.[47] The mechanism regulating tumor elasticity should be further investigated for future elasticity target therapy. Next, we would like to understand the biological features and reactivity of fibroblasts across the whole body to determine groups of fibroblasts with high reactivity to cancer stimuli and high promotion ability. Such a comprehensive study could develop a sub-classification of fibroblasts. Previously, Th3 and Th4 sub-classification in T cells or M1 and M2 sub-classification in macrophages drastically facilitated research in immunology.[48,49] Research in fibroblasts is still in an immature state before the establishment of adequate classification. Lack of specific fibroblast biomarkers makes even its definition elusive. However, this also means potential for future development. Previous studies elucidated some special fibroblasts within the body. However, future study may not just identify a special fibroblast, but will be more comprehensive and establish groups of fibroblasts with special functions and relevant biomarkers. Such studies may use a clear definition and functional classification for fibroblasts that will allow for a better understanding of a wide range of physiological and pathological phenomena. Adequate classification of fibroblasts based on detailed data may provide an initial step to accelerate biological studies and future therapy targeted to fibroblasts.

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DISCLOSURE STATEMENT

None declared.

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