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| 著者 Author(s) | Yokozaki, Hiroshi / Koma, Yu-ichiro / Shigeoka, Manabu / Nishio, Mari |
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Review Article

Cancer as a tissue: The significance of cancer-stromal interactions in the development, morphogenesis and progression of human upper digestive tract cancer

Hiroshi Yokozaki, Yu-ichiro Koma, Manabu Shigeoka and Mari Nishio

Division of Pathology, Department of Pathology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Japan

We review the significance of cancer-stromal interactions (CSIs) in the development, morphogenesis and progression of human gastric and esophageal cancer based on the data obtained from co-culture experiments. Orthotopic fibroblasts in the gastric cancer stroma not only promoted their growth by cancer cells but were also responsible for the mobility, morphogenesis and epithelial-to-mesenchymal transition (EMT) of the cancer cells through CSI. Bone marrow-derived mesenchymal stem cells could be part of the origin of cancer-associated fibroblasts (CAFs) of the gastric cancer providing an advantageous microenvironment for the restoration of cancer stem cells with the induction of the EMT. Tumor-associated macrophages (TAMs) may differentiate from bone marrow-derived monocytes/macrophages within the tumor microenvironment of esophageal cancer and participate in the growth and the progression of esophageal squamous cell carcinomas (ESCCs). Macrophages infiltrated into the intraepithelial neoplastic lesions of the esophagus may function as a biological promoter by promoting the growth and motility of squamous epithelia. Tumor cells build up “cancer as a tissue” by taking advantage of the existing network of growth factors, cytokines and chemokines through the interactions of TAMs, CAFs and cancer cells themselves.

Key words: cancer-associated fibroblast, esophageal cancer, stomach cancer, tumor-associated macrophage, tumor microenvironment

Molecular biological explorations of cancer cells have unraveled that genetic and epigenetic alterations of multiple cancer-related molecules are implicated in their development and progression. Hanahan and Weinberg proposed that the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis.¹

We have attempted to identify factors predicting the biological malignancy of gastrointestinal tract cancers which could be applied to the routine pathological diagnoses of these cancers. Our efforts revealed that the loss of tight junction molecules,²–⁵ and the overexpression of PRL3 phosphatase⁶–¹⁰ as well as that of EZH2¹¹ (a component of polycomb complex) are closely associated with the invasion and metastasis of human gastrointestinal tract cancers. Interestingly, alterations of each molecule were very closely associated with the epithelial-to-mesenchymal transition (EMT) of the cancer cells,⁵,¹²–¹⁴ and the expression of EMT phenotypes was frequently confirmed at the invasive front of the cancer. These observations implied that cancer cells and stroma interact with each other, comprising the tumor microenvironment and defining the malignant phenotype.

The stromal component of the tumor microenvironment consists of fibroblastic cells, vascular endothelial cells, infiltrating immune cells and extracellular matrix (ECM). Cytokines, chemokines, growth factors and enzymes for the matrix remodeling from cancer cells and stroma build up complex networks for their communications which may often provide tumor-promoting function by modulating most of the hallmarks of cancer at all stages of carcinogenesis.¹⁵,¹⁶

Electron-microscopic observations of gastrointestinal cancer tissue demonstrate the frequent close contact of cancer
cells and stromal cells without intervening matrix. This is not a physiological condition. For the elucidation of the pathological significance of cancer-stromal interactions (CSIs), molecular pathological extractions of the molecules responsible for the interactions have been conducted. These extractions have been accomplished by developing several co-culture models of cancer cells and stromal cells, followed by their morphological verification in the actual human cancer tissues. The results of the research concerning the roles of CSIs in the progression, morphogenesis and early carcinogenesis of upper digestive tract cancer will be presented and discussed in this review. The significance of the current understanding of cancer “as a tissue” will be emphasized.

INTERACTION BETWEEN CANCER CELLS AND FIBROBLASTS IN THE MORPHOGENESIS AND PROGRESSION OF GASTRIC CARCINOMAS

Cancer-associated fibroblasts (CAFs)

Cancer-associated fibroblasts are defined as all fibroblasts associated with tumors.17 They are activated within the tumor microenvironment and have been reported to not only provide mechanical support but also to regulate cell growth and survival, angiogenesis, metastasis, immunogenicity and resistance to therapies.18 CAFs express several markers distinguishing them from resting or quiescent fibroblasts, these markers include alpha smooth muscle actin (αSMA) and fibroblast activation protein (FAP).19,20 Fibroblasts expressing αSMA are usually called myofibroblasts, a major population of CAFs. The origin of CAFs have been shown to be heterologous — including local infiltrating fibroblasts, endothelial cells and components of vascular wall, mesenchymal stem cells (MSCs), and cancer cells themselves — reflecting their functional diversity.21,22

Direct or indirect co-culture of gastric cancer cells and orthotopic fibroblasts

As a first-step of the analysis of CSIs, a co-culture system separating cancer and stromal cells with 0.1% collagen gel was developed. TMK-123 cells, a line of poorly differentiated gastric cancer (GC) cells, showed marked scattering on collagen gel when co-cultured with stomach derived fibroblasts, whereas well-differentiated type MKN-2824,25 cells exhibited tubular formation within the collagen gel by fibroblast co-culture via a hepatocellular growth factor/MET paracrine loop.26,27

Next, a direct co-culture system (Fig. 1a) with adherent fibroblasts and floating HSC-39 diffuse-type GC cells, established from the ascites of a diffuse-type GC patient. Experiments with this system demonstrated that orthotopic (gastric), but not heterotopic (intestinal), fibroblasts were directly contacted with HSC-39 and promoted their growth through the activation of integrin α4 signaling by upregulating vascular cell adhesion molecule 1 (VCAM1) (Fig. 1b–e) and inducing the EMT phenotype in HSC-39 cells (Fig. 1f). VCAM1 immunoreactivity was frequently observed in the stromal cells in the diffuse-type GC tissue.29 Serum-free conditioned medium from orthotopic fibroblasts was reported to promote the growth of cancer cells from diffuse-type GC through fibroblast growth factor 7 (FGF7).30,31 These findings suggested that direct and indirect interaction between cancer cells and orthotopic fibroblasts as the cancer-associated fibroblasts (CAFs) may explain the molecular pathological bases of the characteristic growth morphology of diffuse-type GC (Fig. 1g).

Bone marrow-derived mesenchymal stem cells (BM-MSCs) can alter the phenotype of gastric cancer cells with a direct interaction

Among the origins of CAFs described above, MSCs have been studied in regard to the similarity of their gene expression profiles to those of CAFs.32,33 Using a bone marrow transplantation/transfer model, Ishii et al. confirmed that bone marrow cells were recruited into human cancer xenografts and expressed αSMA in severe immune-deficient mice.34,35 Bone marrow-derived myofibroblasts contributed to the MSC niche and promoted tumor growth in mouse models of inflammation-induced gastric cancer.36

To test the possibility of the function of bone marrow-derived MSCs (BM-MSCs) as one of the origins of CAFs in the gastric cancer microenvironment, the GC cell line MKN-724,25 was co-cultured directly with an immortalized human BM-MSC line UE6E7T-12.37 Intriguingly, the MKN-7 cells attached directly to the BM-MSCs, which promoted the cells’ growth and induced the EMT phenotype and the expression of CD133, a marker of cancer stem cell (CSC) (Fig. 2a–d). WNT5A and transforming growth factor beta (TGFβ), factors that were predicted by microarray analyses to be involved in the restoration of stemness in MKN-7 cells, induced CD133 in CD133-negative MKN-7 cells (Fig. 2e–g).38 The transduction of exogenous WNT5A expression into MKN-7 cells upregulated genes related to the EMT and the CSCs. The WNT5A transfectant showed high tumorigenicity in vivo.39 BM-MSCs may mediate the activation of WNT and TGFβ signaling to provide the advantageous microenvironments for the reacquisition and maintenance of CSCs (Fig. 2e). The interactions between BM-MSC-derived...
Orthotopic fibroblast proliferation and the enhancement of invasive properties of cancer cells derived from diffuse-type gastric cancer (GC) were promoted by direct cancer stromal interaction. (a) Schematic presentation of the co-culture system between HSC-39 diffuse-type GC cells and fibroblasts with the cDNA microarray analysis strategy. (b) The growth of the “orthotopic” NF-25 fibroblasts from gastric wall was promoted by the direct contact co-culture with HSC cells. NF-j2, “heterotopic” fibroblasts from jejunal wall. (c) A specific induction of VCAM1 in NF-25 fibroblasts was observed when these cells were co-cultured with HSC-39 diffuse-type GC cells. HSC-57 and HSC-64: non-diffuse-type GC cells. (d,e) VCAM1 and integrin-α4 were both involved in the growth promotion of NF-25 fibroblasts co-cultured with HSC-39 cells. Neutralizing antibodies to VCAM1 (d) and integrin-α4 (e) were added to the co-culture media. IgG, non-specific mouse IgG as a negative control. Each antibody effectively suppressed the growth-promoting effect of co-culture in a dose-dependent manner. (f) Suppression of adhesion molecules in NF-25 fibroblasts and the induction of EMT-like molecular changes in HSC-39 diffuse-type GC cells by direct co-culture (upper panel, Western blot). Up-regulation of MMPs in HSC-39 cells by NF-25 co-culture (lower panel, RT-PCR). (g) A direct contact interaction between orthotopic fibroblasts and diffuse-type GC cells may induce growth in the former and the EMT-like phenotype in the latter making up the characteristic tissue morphology as scirrhus carcinoma. (Reproduced and modified from Semba S, Kodama Y, Ohnuma K, Mizuuchi E, Masuda R, Yashiro M, Hirakawa K, Yokozaki H. Direct cancer-stromal interaction increases fibroblast proliferation and enhances invasive properties of scirrhus-type gastric carcinoma cells. Br J Cancer 2009; 101: 1365–73. DOI: 10.1038/sj.bjc.6605309).
Figure 2  Bone marrow derived mesenchymal stem cells (BM-MSCs) provide an advantageous tumor microenvironment for the restoration of cancer stem cell properties.  

(a) The growth of MKN-7 GC cells was promoted by UE6E7T-12 BM-MSCs by direct contact co-culture, whereas conditioned media of UE6E7T-12 (UE6E7T-12 sup.) did not have any effect on the growth of MKN-7 cells.  

(b) Aggregation assay in soft agar plates after 72 h of co-culture demonstrated the formation of a tumor sphere-like structure of MKN-7 cells surrounding a GFP-labelled UE6E7T-12 BM-MSCs (lower left panel) as a core (upper left panel).  

(c) Direct co-culture of MKN-7 cells with UE6E7T-12 BM-MSCs induced EMT-like (decreased CDH1, increased VIM and SNAI1) and cancer stem cell-like (increased CD44 and CD133) gene expressions.  

(d) The population of CD133+ MKN-7 cells was increased by direct contact with UE6E7T-12 BM-MSCs. CD133+ and CD133-MKN-7 cells were maintained for 48 h in the presence or absence of UE6E7T-12 BM-MSCs, and then the isolated MKN-7 cells were divided into CD133+ and CD133-cells. Cell separation was performed by the magnetic beads method using an autoMACS Pro separator.  

(e) The expression of five representative up-regulated genes (WNT5A, TGFBI, SNAI2, AKT3 and ID3) extracted by cDNA microarray. The mRNAs were prepared from MKN-7 cells: left panel, co-culture (+) vs co-culture (−); right panel, CD133+ vs CD133-MKN-7 cells. Quantitative RT-PCR assay.  

(f) Treatment with recombinant WNT5A (500 ng/mL) and TGFβ1 (10 ng/mL) induced CD133 expression in MKN-7 cells. Recombinant WNT3A (500 ng/mL) was used as a control.  

(g) The population of CD133+ MKN-7 cells was also increased by WNT5A and TGFβ1 in vitro. MKN-7 cells were divided into CD133+ and CD133-cells after 48 h treatment by the magnetic bead method. WNT3A, a control.  

(h) A schematic model of the cell-cell interaction between GC cells and BM-MSCs. (Reproduced and modified from Nishimura K, Semba S, Aoyagi K, Sasaki H, Yokozaki H. Mesenchymal stem cells provide an advantageous tumor microenvironment for the restoration of cancer stem cells. Pathobiology 2012; 79: 290–306. DOI: 10.1159/000337296).
CAFs and other components of cancer stroma will be presented and discussed later in this review.

OUR UNDERSTANDING OF CANCER TISSUE ON THE BASES OF THE INTERACTIONS BETWEEN TUMOR-ASSOCIATED MACROPHAGES AND CANCER CELLS

Macrophage polarization and tumor-associated macrophages (TAMs)

The origin, differentiation and roles in the physiological and pathological conditions of macrophage have been elucidated by many investigators. Bone marrow-derived peripheral blood monocytes (PBMos) are recruited through the blood vessels and differentiate into macrophages by various pathological stimuli at the peripheral tissue. Macrophages are known to be activated into pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes depending on the local microenvironment of the diseased tissue. Macrophages with M1 activation express high levels of pro-inflammatory cytokines (interleukin [IL]1, IL-6, IL12 and tumor necrosis factor alpha [TNFα]). In contrast, M2 macrophages demonstrate an IL4/IL10high and IL12low cytokine expression profile, secrete factors associated with tissue remodeling (vascular endothelial growth factor A [VEGFA], matrix metalloproteinase [MMP]2 and MMP9) and express cell surface markers such as mannose receptor and scavenger receptors (CD163, CD204). From the oncological viewpoints, M1 macrophages are tumor-suppressive and those with M2 type are tumor-supportive in the functional nature.

Rudolf Virchow initially described that many leukocytes including macrophages were found in the neoplastic tissues. It is well known that he drew the connection between inflammation and tumorigenesis from this observation. Leukocytes including macrophages have been considered to participate in the host defense mechanisms. However, since the recent development of monoclonal antibodies to the specific differentiation surface markers established the identification of macrophages in the tumor tissues, it has been elucidated that the infiltrated numbers or the densities of macrophages — especially those with M2 phenotypes — are closely associated with the tumor progression and poor prognosis of the patients in many human malignancies.

CD204-positive TAMs in esophageal squamous cell carcinomas (ESCCs) was associated with tumor aggressiveness

We have been investigated the roles of macrophages in the ESCCs and made the first report of the close association of these tumors’ aggressiveness and the infiltrating number of CD204-positive macrophages. We detected CD68-, CD163- or CD204- positive macrophages in all of the cancer tissues of 70 ESCCs (Fig. 3a). We calculated the microvessel density (MVD) by identifying the highly CD34-positive vascular areas in each ESCC tissue (Fig. 3b) and counted the macrophage number in three independent high-power microscopic fields of cancer nests within the areas of the microvessel count. A significant correlation was also observed between the MVD and the number of CD204-positive macrophages (Fig. 3c). A shown in Table 1, when we used the median macrophage number to divide the ESCC cases into high number and low number groups, the cases with a high number of CD204-positive macrophages demonstrated a significant correlation with more malignant phenotypes (i.e., regarding the depth of tumor invasion, lymphatic and blood vessel infiltration, lymph node metastasis and stages) as well as poor disease-free survival (Fig. 3d).

Cysteine rich angiogenic factor 61 (CYR61) was induced in TAM-like cells derived from THP-1 human monocytic leukemia cell line THP-1

As the clinical research on ESCC cases suggested the roles of CD204-positive TAMs in the progression and angiogenesis of these tumors, we exposed a human monocytic leukemia-derived cell line (THP-1) treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) to the conditioned media (CM) of several TE-series ESCC cell lines (TECM) in order to examine the specific differentiation of macrophages to the M2 phenotype (Fig. 3e).

Interestingly, the TECM of five different ESCC cell lines (TE-8, TE-9, TE-10, TE-11, TE-15) induced CD204 and VEGFA expression in TPA-treated THP-1 cells, without any exception (Fig. 3e,f). An immediate-early gene CYR61 was extracted as one of the induced genes during the M2 differentiation of THP-1 cells by exposure to TECM. CYR61 is an ECM component and induces angiogenesis by stimulating the mobility of endothelial cells via αVβ3 integrin. The growth and progression of various cancers including esophageal cancer has been linked to the over-expression of CYR61.

Our research demonstrated that CYR61 is expressed not only in cancer cells but also in TAMs showing close association with the infiltrated number of CD204-positive macrophages in ESCC tissues. TPA-treated macrophage-like THP-1 cells showed enhanced migration and induction of the expression of CD204 via the activation of MEK/Erk pathway by recombinant human CYR61.
Figure 3  CD204-expressing macrophages are induced in the tumor microenvironment and are associated with the biological malignancy of esophageal squamous cell carcinoma (ESCC). (a) CD204-positive macrophages in the ESCC. Bar: 50 μm. (b) CD34-positive microvessels in the serial section of panel (a). The mean number of microvessels per 0.7386 mm² was calculated as the microvessel density (MVD). Bar: 50 μm. (c) MVD was significantly higher in the ESCCs with a high number of CD204-positive macrophages. The median macrophage number in the cancer nests within the areas of the microvessel count was used to divide the patients into high-number and low-number groups. *P < 0.01. Student’s t-test. Bars: mean ± SEM. (d) The disease-free survival after curative surgery was significantly poor in the ESCC cases with a high number compared to the cases with a low number of CD204-positive macrophages. Kaplan-Meier analysis. *P < 0.05. (e) THP-1 cells were treated with 200 nM TPA for 2 days to induce macrophage-like differentiation, then exposed to 50% conditioned media of TE series esophageal cancer cell lines (TECMs) for 2 days (upper panel). An RT-PCR analysis demonstrated the induction of CD204 in THP-1 cells by every TECM (lower panel). (f) A quantitative RT-PCR showed that the expression of VEGFA was significantly induced by all TECMs tested. *P < 0.05. Student’s t-test. Bars: mean ± SEM. (g) The Transwell migration of TPA-treated macrophages (macrophage-like THP-1) was significantly promoted by recombinant human CYR61 (rhCYR61), and was effectively suppressed by U0126. (h) The induction of CD204 in macrophage-like THP-1 cells by rhCYR61 which was also suppressed by U0126 (Western blot). (i) Pretreatment of macrophage-like THP-1 cells with U0126 inhibited the phosphorylation of Erk1/2 induced by rhCYR61. (Reproduced and modified from Shigeoka M, Urakawa N, Nakamura T, Nishio M, Watajima T, Kuroda D, Komori T, Kakeji Y, Semba S, Yokozaki H. Tumor associated macrophage expressing CD204 is associated with tumor aggressiveness of esophageal squamous cell carcinoma. Cancer Sci 2013; 104: 1112–9. DOI: 10.1111/cas.12188 and Shigeoka M, Urakawa N, Nishio M, Takase N, Utsunomiya S, Akiyama H, Kakeji Y, Komori T, Koma Y, Yokozaki H. Cyr61 promotes CD204 expression and the migration of macrophages via MEK/ERK pathway in esophageal squamous cell carcinoma. Cancer Med 2015; 4: 437–46. DOI: 10.1002/cam4.401).
TECM also induced M2 characteristics in peripheral blood monocyte derived macrophages

Based on the results of the initial investigations described above, we hypothesized that TAMs (mainly with CD204 expression) may have significant roles in the progression of ESCCs whose microenvironment might lead to the specific differentiation of macrophages derived from PBMos. To test this hypothesis, we isolated CD14-positive cells from the buffy-coat fraction of peripheral blood from several healthy volunteers using an autoMACS Pro separator. After the PBMos were incubated with colony stimulating factor 1 (CSF1) for 6 days to induce differentiation into macrophages, TECMs were applied for 2 days (Fig. 4a). A morphological examination confirmed that PBMos elongate their cytoplasm after macrophage-like differentiation with CSF1 stimulation, and the enlargement of both the cytoplasm and nucleus took place in TAM-like cells with the exposure to all five TECMs. The expression of CD163 and CD204 scavenger receptors was induced in TAM-like cells after TECM exposure in comparison with macrophage-like cells (Fig. 4b–d). TAM-like cells exhibited IL10high/IL12low cytokine profile (Fig. 4e,f). The expression levels of VEGFA, MMP2 and MMP9 (characteristic cytokines secreted from M2 macrophages) were higher in the TAM-like cells than in the macrophage-like cells (Fig. 4g–i). These observations confirmed that M2-like differentiation was induced in PBMo-derived macrophage-like cells exposed to TECM (TAM-like cells).

The induction of TAM-like differentiation of PBMo-derived macrophages has been reported in other human malignancies. Tumor-derived IL4, IL10, TGFβ, CSF1 and lactic acid have been considered to have a function in the M2-like polarization of TAMs. Kaneda et al. demonstrated that macrophage PI3-kinase γ controlled a critical switch between immune stimulation and suppression during inflammation and cancer. To explore the molecules specifically regulated during the TAM-like differentiation of macrophages

| Table 1 Tumor infiltrating CD204-positive macrophages in esophageal squamous cell carcinomas and their correlation with clinicopathological parameters |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Number of cases | Low (n = 34)    | High (n = 36)   | P-value‡         |
| **Age**                        |                 |                 |                 |                 |
| <65                            | 33              | 15              | 18              | 0.6408          |
| ≥65                            | 37              | 19              | 18              |                 |
| **Histological grade§**        |                 |                 |                 |                 |
| HGIEN                          | 4               | 4               | 0               | 0.0380*         |
| WDSCC                          | 12              | 6               | 6               |                 |
| MDSCC                          | 43              | 22              | 21              |                 |
| PDSCC                          | 11              | 2               | 9               |                 |
| **Depth of invasion§**         |                 |                 |                 |                 |
| T1a                            | 19              | 18              | 1               | 0.0001*         |
| T1b                            | 30              | 14              | 16              |                 |
| T2 + T3                        | 21              | 2               | 19              |                 |
| **Lymphatic vessel invasion§** |                 |                 |                 |                 |
| Negative                       | 37              | 26              | 11              | 0.0001*         |
| Positive                       | 33              | 8               | 25              |                 |
| **Blood vessel invasion§**     |                 |                 |                 |                 |
| Negative                       | 43              | 28              | 15              | 0.0006*         |
| Positive                       | 27              | 6               | 21              |                 |
| **Lymph node metastasis§**     |                 |                 |                 |                 |
| Negative                       | 43              | 29              | 14              | <0.0001*        |
| Positive                       | 27              | 5               | 22              |                 |
| **Stage**                      |                 |                 |                 |                 |
| 0 + I                          | 38              | 27              | 11              | <0.0001*        |
| II + III + IV                  | 32              | 7               | 25              |                 |

†The median value of CD204-positive macrophage number of cancer nest within the areas of microvessel count was used to divide the patients into high and low groups.
‡Data were analyzed by χ²-test and *P < 0.05 was considered statistically significant.
§According to the Japanese Classification of Esophageal Cancer. HGIEN, high grade intraepithelial neoplasia; WDSCC, well-differentiated squamous cell carcinoma; MDSCC, moderately differentiated squamous cell carcinoma; PDSCC, poorly differentiated squamous cell carcinoma. T1a, tumor invades mucosa; T1b, tumor invades submucosa; T2, tumor invades muscularis propria; T3, tumor invades adventitia.

According to the TNM classification by UICC.

Reproduced and modified from Shigeoka M, Urakawa N, Nakamura T, Nishio M, Watajima T, Kuroda D, Komori T, Kakeji Y, Sembas S, Yokozaki H. Tumor associated macrophage expressing CD204 is associated with tumor aggressiveness of esophageal squamous cell carcinoma. Cancer Sci 2013; 104: 1112–9. DOI: 10.1111/cas.12188.
Figure 4 M2-like characteristics are induced by conditioned media of ESCC cell lines in peripheral blood monocyte (PBMo)-derived macrophages. (a) To induce macrophage-like differentiation, CSF1 was applied to the PBMo for 6 days, the cells were exposed to TECM for 6 days. (b) TECMs (TE-8CM, TE-9CM and TE-15CM) induced the expression of CD163 (upper panels) and CD204 (lower panels) in PBMo-derived macrophages by immunofluorescence. (c,d) A qRT-PCR demonstrated that the induction of mRNA expression of CD163 (c) and CD204 (d) was significant in TECM-treated PBMo-derived macrophages than those without TECM exposure. *P < 0.05, Student's t-test. Bars: mean ± SEM. (e,f) TECM treatment induced an IL10 high (e) and IL12 low (f) M2-type cytokine expression profile in PBMo-derived macrophages. qRT-PCR, *P < 0.05, Student's t-test. Bars: mean ± SEM. (g–i) Expressions of VEGFA (g), MMP2 (h) and MMP9 (i), characteristic cytokines for M2 differentiation, were induced in TECM treated PBMo-derived macrophages. qRT-PCR, *P < 0.05, Student's t-test. Bars: mean ± SEM. (Reproduced and modified from Urakawa N, Utsunomiya S, Nishio M, Shigeoka M, Takase N, Arai N, Kakeji Y, Koma Y, Yokozaki H. GDF15 derived from both tumor-associated macrophages and esophageal squamous cell carcinomas contributes to tumor progression via Akt and Erk pathways. Lab Invest 2015; 95: 491–503. DOI: 10.1038/labinvest.2015.36 and Nishio M, Urakawa N, Shigeoka M, Takase N, Ichihara Y, Arai N, Koma Y, Yokozaki H. Software-assisted morphometric and phenotype analyses of human peripheral blood monocyte-derived macrophages induced by a microenvironment model of human esophageal squamous cell carcinoma. Pathol Int 2016; 66: 86–93. DOI: 10.1111/pin.12381).
as described above, we performed cDNA microarray analyses. The detailed microarray data can be found in the Gene Expression Omnibus repository (GSE59948). Among the molecules induced during TAM-like differentiation, in this review we will discuss growth differentiation factor 15 (GDF15), neural cell adhesion molecule 1 (NCAM1) and chemokines including C-X-C motif chemokine ligand 8 (CXCL8) and C-C motif chemokine ligand 3 (CCL3).

The TGFβ family growth factor GDF15, promoted the growth, migration and invasion of ESCC cell

GDF15 is a distant member of the bone morphogenetic protein (BMP) subfamily of the TGFβ superfamily growth factor.64 It is also known as macrophage-inhibiting cytokine 1 (MIC1),65 non-steroidal anti-inflammatory drug-activated gene (NAG1),66 prostate-derived factor (PDF),57 bone morphogenetic protein, placenta (PLAB),68 and placental TGFβ (PTGFβ).69 GDF15 is induced by hypoxia and acute tissue injury, and it acts as an autocrine activator of macrophage during the process of inflammation. Overexpression of GDF15 has been reported to be associated with the growth, survival and invasion of various cancers.70-72 Its true receptor has not been identified.

An induced secretion of GDF15 from TECM-exposed TAM-like cells in comparison with macrophage-like cells was confirmed by an enzyme-linked immunosorbent assay (ELISA) (Fig. 5a). TE-9 also secreted significant levels of GDF15 (Fig. 5a). Recombinant human GDF15 promoted the growth (Fig. 5b), Transwell migration and Matrigel invasion of ESCC cells (S. Utsunomiya, M. Okamoto, unpubl. data, 2017). As it has been reported that PI3K/Akt and MEK/Erk signaling pathways were activated by GDF15,70,71 the phosphorylation status after an administration of recombinant GDF15 in TE cells was analyzed by Western blots. GDF15 enhanced the phosphorylation of Akt (Ser473 and Thr308) and Erk1/2 (Thr202/Tyr204) after 10 min of GDF15 exposure (Fig. 5c). Pretreatment with a PI3K inhibitor (LY294002) or a MEK1/2 inhibitor (PD98059) suppressed the GDF15-induced growth of ESCC cells (Fig. 5d).

Immunofluorescence demonstrated that positive GDF15 signals were present both in cancer nests and in stroma with co-localization of CD204-positive TAMs (Fig. 5e). When the immunoreactivity in 70 ESCC tissue samples was divided into high and low immunoreactivity in comparison with corresponding normal esophageal squamous epithelia (Fig. 5f, GDF15 high), the tissue samples with high expression were significantly correlated with depth of tumor invasion, lymph and blood vessel invasion, lymph node metastasis, stage and the infiltrated number of macrophages.57 Moreover, ESCC patients with high-GDF15 expression tumors demonstrated significantly shorter disease-free survival and overall survival compared to the patients with low-GDF15 tumors (Fig. 5g,h).

NCAM1, induced in TAM-like cells, enhanced FGF2/FGFR1 signaling by co-localizing with FGFR1 and promoted the cells’ survival and migration

NCAM1 is a member of the immunoglobulin superfAMILY glycoprotein initially discovered as a homophilic adhesion molecule of chick nerve retina cells.74 NCAM1 is also expressed by hematopoietic cells including NK cells, a subset of T cells, plasma cells and a minor population of peripheral blood monocytes.75 NCAM1 also interacts heterophilically with several cell membrane-associated molecules including FGF receptor 1 (FGFR1) and regulates the intracellular signaling.76 NCAM1 was up-regulated in TAM-like cells after exposure to the conditioned media of TE-8, TE-9 and TE-15 ESCC cell lines (TAM9, TAM8 and TAM15, respectively) and co-localized with F-actin at a part of the lamellipodia suggesting its functional involvement in cell mobility.77 Moreover, NCAM1 was confirmed to be co-localized with FGFR1 (Fig. 6a) the phosphorylation status of which was suppressed by the silencing of NCAM1 in TAM9 (Fig. 6b). FGF2 was also up-regulated in TAM-like macrophages, and an enhanced phosphorylation of FGFR1 was observed in TAM9 and TAM15 (Fig. 6c). Recombinant FGF2 promoted Transwell migration and cell survival of TAM9 which was significantly inhibited by a FGFR inhibitor SU5402 (Fig. 6d,e). FGF2 induced the phosphorylation of Akt (Ser473 and Thr308), MEK1/2 and Erk1/2 after 10 min of treatment (Fig. 6f) which was also significantly inhibited by the selective FGFR1 inhibitor SU5402 (Fig. 6g). These results suggested that NCAM1 by associating with and regulating the phosphorylation status of FGFR1, enhanced FGF2 signaling via the activation of PI3K-Akt and MEK1/2-Erk1/2 in TAM-like macrophages. ESCC cells but not TAM-like macrophages secrete a detectable amount of FGF2 into culture media, whereas a positive FGF2 signal was observed in TAM-like cells by immunofluorescence (Fig. 6h). This may exhibit the FGF2/FGFR1 intracrine mechanisms reported in pancreatic stellate cells facilitating pancreatic cancer cell invasion.78

ESCC cells also expressed FGFR1 and promoted survival and migration by exogenous FGF2 through PI3K-Akt and MEK1/2-Erk1/2 activation (Fig. 6i-1). Immunofluorescence of ESCC tissue demonstrated that FGF2 was expressed not only in cancer cells but also in stromal cells some of which co-expressed CD204 (Fig. 6m).

The hypothesized interactions of cancer cells and macrophages in the microenvironment of ESCC tissue mediated by CYR61, GDF15 and NCAM1 are summarized in Figure 7.
Figure 5  Roles of GDF15 derived from both tumor-associated macrophages and ESCCs in tumor progression. (a) GDF15 concentration in the culture media of TECM exposed PBMo-derived macrophages and ESCC cell lines measured by ELISA. TECM induced significant GDF15 secretion from TECM exposed macrophages. The TE-9 ESCC cell line also produced a significant amount of GDF15. **P < 0.01, Student’s t-test. Bars: mean ± SEM. (b) Exogenous GDF15 (rhGDF15) promoted the growth of the TE-8 ESCC cells in a dose-dependent manner. A significant difference was observed between NT (no treatment) and 100 ng/mL rhGDF15. **P < 0.01, Student’s t-test. Bars: mean ± SEM. (c) Effect of rhGDF15 on the phosphorylation of Akt and Erk1/2. Western blot results demonstrated that both Erk1/2 and Akt were activated after 10 min of rhGDF15 treatment in TE-8 cells. **P < 0.01, Student’s t-test. Bars: mean ± SEM. (d) The MTS assay results demonstrated that pretreatment with LY294002 (a PI3K inhibitor) or PD98059 (a MEK inhibitor) suppressed the GDF15-promoted growth of TE-8 cells. (e) The immunofluorescence of ESCC tissue with anti-GDF15 (green) and anti-CD204 (red) antibodies demonstrated GDF15 signals in both cancer nests and stroma. CD204 and GDF15-positive macrophages are indicated by arrows. Nuclei were stained with DAPI (blue). Bars: 20 μm. (f) An ESCC tissue exhibiting high GDF15 immunoreactivity in comparison with corresponding normal esophageal mucosa. Bar: 100 μm. (g,h) The patients with GDF15-high ESCC had significantly poorer disease-free (g) and overall (h) survival compared to those with GDF15-low tumors. *P < 0.05, Kaplan-Meier analysis. (Reproduced and modified from Urakawa N, Utsunomiya S, Nishio M, Shigeoka M, Takase N, Arai N, Kakeji Y, Koma Y, Yokozaki H. GDF15 derived from both tumor-associated macrophages and esophageal squamous cell carcinomas contributes to tumor progression via Akt and Erk pathways. Lab Invest 2015; 95: 491–503. DOI: 10.1038/labinvest.2015.36).
NCAM1- and FGF2-mediated FGFR1 signaling in the esophageal cancer tumor microenvironment regulates the survival and migration of tumor-associated macrophages and cancer cells. (a) Double immuno fluorescence demonstrated the co-localization of the signals of NCAM1 (green) and FGFR1 (red) mainly at the cell membranes of PBMo-derived macrophages treated with TE-9CM (TAM9 cells). Bar: 10 μm. (b) The expression as well as phosphorylation of FGFR1 was suppressed by the silencing of NCAM1 in TAM9 cells. Western blot analysis. (c) Western blot results demonstrated that both the expression and the phosphorylation level were induced in PBMo-derived macrophages treated with a TECM (TE-8, TE-9 and TE-15). (d,e) FGF2 promoted the Transwell migration (d) and the survival (e) of TAM9 cells both of which were significantly suppressed by SU5402, a selective FGFR1 inhibitor. (f) Exogenous FGF2 induced the phosphorylation of Akt, MEK1/2 and Erk1/2 in TAM9 cells after 10 min of treatment. Western blot. (g) The FGFR1 inhibitor SU5402 suppressed the FGF2-activated PI3K-Akt and MEK1/2-Erk1/2 pathway in TAM9 cells. Western blot. (h) ELISA results demonstrated that a significant amount of FGF2 was detected in the culture media of ESCC cells (TE-8, TE-9 and TE-15) but not in that of TAM-like macrophages. However, FGF2 signals (green) were detected in CD204 (red)-positive TAM9 cells (inset). Bar: 10 μm. (i) Western blot results confirmed the expression of FGFR1 in the ESCC cell lines. (j) Exogenous FGF2 induced the phosphorylation of Akt, MEK1/2 and Erk1/2 in TE-9 cells after 10 min of treatment. FGFR1 inhibitor SU5402 suppressed the FGF2-activated PI3K-Akt and MEK1/2-Erk1/2 pathway in TE-9 cells. Western blot. (k) FGF2 promoted the Transwell migration (k) and the survival (l) of TE-9 cells, and both the migration and survival were significantly suppressed by the selective FGFR1 inhibitor SU5402. *P < 0.05, **P < 0.01, Student’s t-test. Bars: mean ± SEM. (m) The immunofluorescence of ESCC tissue with anti-FGF2 (green) and anti-CD204 (red) antibodies demonstrates FGF2 signals in both cancer nests and stroma. CD204- and FGF2-positive macrophages are indicated by arrowheads. Nuclei were stained with DAPI (blue). Bar: 50 μm. (Reproduced and modified from Takase N, Koma Y, Urakawa N, Nishio M, Arai N, Akiyama H, Shigeoka M, Kakeji Y, Yokozaki H. NCAM- and FGF-2-mediated FGFR1 signaling in the tumor microenvironment of esophageal cancer regulates the survival and migration of tumor-associated macrophages and cancer cells. Cancer Lett 2016; 380: 47–58. DOI: 10.1016/j.canlet.2016.06.009).
PBMs are recruited from blood vessels and activated into macrophages, and then TAMs within the ESCC microenvironment. CYR61, induced in TAMs and derived from cancer cells, induces CD204 expression and the migration of macrophages by activating MEK-Erk signaling. GDF15, also induced in TAMs and derived from cancer cells, promotes the growth, migration and invasion of cancer cells through the activation of Akt and Erk pathways. FGF2 from cancer cells not only promotes the cells’ survival and migration but also enhances the survival and migration of TAMs through NCAM1-reinforced classical FGFR1 signaling as well as by intracrine FGF2/FGFR1 signaling by activating Akt and Erk pathways.

Chemokines constituted multiple paracrine and autocrine loops for the promotion of the migration and invasion of cancer cells and monocytes/macrophages in the ESCC microenvironment

Chemokines are the low-molecular-weight cytokines regulating the chemotactic movement of leukocytes. They are single strand polypeptides that consist of 70—100 amino acids sharing 20%—95% sequence homologies to each other. Chemokine receptors are rhodopsin-like, seven-transmembrane, G protein-coupled single polypeptide receptors that also having 25%—80% amino acid sequence similarity to each other. After ligand binding, chemokine receptors dissociate into α and βγ subunits. The α subunits activate mainly the PKA and PLC pathways, and the βγ subunits activate mainly the PI3K pathway. Many different chemokines bind the same receptor and many chemokines bind multiple receptors.

CXCL8, also known as IL8, was identified as a neutrophil chemotactic factor secreted from THP-1 cells. CXCL8 is mainly secreted from monocyte/macrophages, endothelial cells and fibroblasts and binds to the receptors, CXCR1 and CXCR2. CXCL8 was reported to be associated with the progression of several human malignancies.

A high expression level of CXCL8 in TAM-like PBM-derived macrophages and the expressions of CXCR1 and CXCR2 in TE-series ESCC cell lines were confirmed. CXCL8 induced the migration and invasion of the ESCC cells by the phosphorylation of Akt and Erk1/2. Indirect cocultures demonstrated that not only inhibitors to PI3K-Akt

Figure 7 Interaction of cancer cells and macrophages through CYR61, GDF15 and NCAM1 in the ESCC tumor microenvironment.

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and MEK1/2-Erk1/2 but also neutralizing antibodies against CXCL8, CXCR1 and CXCR2 suppressed the migration and invasion of ESCC cells induced by TAM-like PBMo-derived macrophages.

Our immunohistochemical analysis of 70 resected ESCC samples showed that high expression levels of CXCL8 in ESCC tissues were significantly associated with lymph node metastasis and poor prognosis. These results suggest that CXCL8 up-regulated in the microenvironment may contribute to ESCC progression by promoting migration and invasion of cancer cells (Fig. 8).86

CXCL8, secreted mainly from monocytes, macrophages and dendritic cells, recruits and activates neutrophils in acute inflammation.87 CXCL8 also recruits monocytes/macrophages, a fraction of T cells and dendritic cells using two main receptors CXCR1 and CXCR2. It has been reported that the CXCL8/CXCR2 axis promoted the lung metastasis of a murine renal cell carcinoma by an intratumoral accumulation of leukocytes and fibroblasts and the promotion of angiogenesis.88

CCL3, secreted mainly from monocytes, macrophages and dendritic cells, recruits and activates neutrophils in acute inflammation.87 CCL3 also recruits monocytes/macrophages, a fraction of T cells and dendritic cells using two main receptors CCR1 and CCR5. It has been reported that the CCL3/CCR5 axis promoted the lung metastasis of a murine renal cell carcinoma by an intratumoral accumulation of leukocytes and fibroblasts and the promotion of angiogenesis.88

CCL3 was induced in PBMo-derived macrophages after TECM exposure. The TAM-like cells secreted significant amount of CCL3 into culture media. The two known receptors for CCL3, i.e., CCR1 and CCR5, were expressed in ESCC cell lines. CCL3 induced the Transwell migration of ESCC cells through activation of PI3K-Akt and MEK1/2-Erk1/2 pathways. Not only PI3K-Akt or MEK1/2-Erk1/2 inhibitors but Maraviroc, a CCR5 antagonist, suppressed the CCL3-induced cell migration of ESCC cell lines (Fig. 8; A. Iijima and N. Arai, unpubl. data, 2016).

CCL2 was discovered as a monocytic chemotactic factor from macrophages.89 Interestingly, cDNA microarray analysis of mono-cultured ESCC cell lines and co-cultured ESCC cell lines with TAM-like cells revealed that CCL2 was also up-regulated in ESCC cells that interacted with TAM. CCR2, a receptor for CCL2, was expressed not only in monocytes/macrophages but also in ESCC cells. CCL2 promoted the migration of the ESCC cell lines through the activation of PI3K-Akt and MEK1/2-Erk1/2 pathways. In addition, exogenous CCL2 administrated to TAM-like cells up-regulated the expression of CCL3 as well as CCL2 itself, suggesting that CCL2 is an autocrine and paracrine inducer of CCL2 and CCL3 in TAMs resulting in the promotion of cancer cell migration in the ESCC microenvironment (Fig. 8; K. Suemune, unpubl. data, 2017).

Significance of the interaction among cancer cells, TAMs and CAFs in the tumor tissue

As discussed earlier, CAFs are also a major component of tumor stroma. There must be cross-talk among CAFs, TAMs and cancer cells within the tumor microenvironment.90

Although Herrera et al. reported the prognostic involvement of interrelationships between markers of CAFs and TAMs in colorectal tissues,91 cell-to-cell interactions of CAFs, TAMs and tumor cells have not been elucidated. We have reported the collaboration of CAFs and TAMs in neuroblastoma as following.92 Our immunohistochemical analysis of CAFs on the bases of the staining area of αSMA and the infiltrating numbers of CD68-, CD163- or CD204-positive TAMs demonstrated their close co-localization and significant association with the clinical stage, MYCN amplification, bone marrow metastasis, histological classification, histological type, and risk classification. The conditioned media of a neuroblastoma cell line also induced TAM-like differentiation in PBMo-derived macrophages. TAM-like cells induced αSMA expression in BM-MSCs, which enhanced the growth of the neuroblastoma cell line. TAM-like cells promoted not only the invasion of neuroblastoma cell line but also the proliferation of CAFs and TAMs.
of BM-MSCs. A cytokine array analysis revealed that CXCL2 secreted from TAM-like macrophages was the significant effector of tumor invasiveness.

We also confirmed that the intensity of αSMA and FAP expression in the stromal fibroblasts was correlated with the clinicopathological features and disease-free survival rates in ESCCs. ESCC cell lines induced αSMA and FAP in BM-MSCs which induced M2 polarization of macrophage-like cells (N. Higashino, unpubl. data, 2017).

**IMPLICATION OF EPITHELIAL AND MACROPHAGE INTERACTION IN THE EARLY STAGE OF ESOPHAGEAL CARCINOGENESIS**

Figure 9a depicts the squamous cell carcinoma in situ (CIS) of the esophagus excised by endoscopic submucosal dissection. Intraepithelial neoplastic epithelial growth is observed on the left side demarcated by the oblique line adjacent to the non-neoplastic squamous epithelium on the right side. The figure's lower panels are of the Ki-67, CD163 and CD204 immunohistochemistry of the serial sections. Notably, the densities of CD163- or CD204-positive cells are much higher in the neoplastic epithelial part with higher Ki-67 labeling (especially around the intraepithelial papillary capillary loops) than in the adjoining non-neoplastic part (compare Fig. 9a-i and 9a-ii, CD204-positive cells in neoplastic and non-neoplastic epithelia, respectively).

These observations implied the possible involvement of macrophages, especially M2 skewed macrophages, in the early stage of the carcinogenesis of squamous epithelia including the support for the growth of neoplastic cells and the induction of abnormal angiogenesis.51,93

The delivery of 4-nitroquinoline 1-oxide (4-NQO) in the drinking water to CBA or C57B1/6 female mice provides a model for the multistep squamous epithelial carcinogenesis of the oral cavity and esophagus.84 In this model, squamous neoplasia including invasive carcinoma was observed from the 6th week after 4-NQO treatment (100 μg/mL) for 16 weeks. The normal, hyperplasia, dysplasia, CIS and invasive squamous cell carcinoma (SCC) tissues were obtained from the esophagus of 4-NQO-exposed animals in the 15th week after the end of the treatment (kindly provided by Dr. Yu Usami, Osaka University). The average number of macrophages that were positive for Iba1 (a macrophage/microglia specific calcium binding protein) per unit area (Iba1+ cells/1 × 10^4 μm^2) was significantly increased in the dysplasia/CIS (3.9 ± 1.52, P < 0.05) and invasive SCC (8.3 ± 2.28, P < 0.01) compared to the normal (1.2 ± 0.77)/hyperplastic (1.4 ± 0.46) epithelia and in the invasive SCC compared to the dysplasia/CIS (P < 0.05) (Y. Koma, Y. Usami, unpubl. data, 2017). Hammes et al. also reported the CD68-positive macrophage infiltration was closely related to the progression of human uterine cervical neoplasia.96

We then investigated the time point at which the macrophage infiltration to pre-neoplastic esophageal epithelia takes place? Amanuma et al. reported observing significantly higher levels of phosphorylated histone H2AX (γ-H2AX; a well-established marker of DNA damage) and N2-ethylidene-2-deoxyguanosine in the esophagus of aldehyde dehydrogenase 2 (Aldh2) knock-out mice compared to wild-type mice after 8 weeks of 10% ethanol water consumption, which suggested that acetaldehyde-derived esophageal DNA damage was enhanced in the absence of Aldh2 gene expression.97

Interestingly, we found that the mean infiltrated number of Iba1-positive macrophages per unit length of mucosa (cells/1 × 10^4 μm) was significantly higher (P < 0.01) in the squamous epithelia of Aldh2 knockout mice (21.9 ± 4.46) compared to the wild-type mice (7.8 ± 5.23) using the same samples (kindly provided by Prof. Manabu Muto and Dr. Yusuke Amanuma, Kyoto University; Y. Koma, Y. Amanuma, M. Muto, unpubl. data, 2017). These results suggested that macrophages may catch the signals from injured epithelia which are not neoplastic yet, and are then recruited toward the damaged focus to establish some interactions.

It has been reported that macrophages secrete epidermal growth factors in breast cancer tissue.98 We confirmed that TGFA was upregulated in activated macrophages and that the activated macrophages promoted the growth and migration of Het-1A cells (a SV40 T-antigen immortalized human esophageal epithelial cell line),99 and induced the expression and secretion of IL6 as well as the activation of p38MAPK signal by co-culture.100 These findings may indicate that macrophages infiltrating into the squamous epithelia responding to some damage signal(s) from the injured epithelia at the initiation stage of esophageal carcinogenesis may stimulate the growth and mobility of epithelial cells to help the establishment of cancer cells (Fig. 9b).

Lastly, we present our working hypothesis on the roles of macrophages that have infiltrated into the pre-neoplastic squamous epithelia in early esophageal carcinogenesis (Fig. 9c). Balkwill and Mantovani suggested that the contribution of inflammatory cells and cytokines to the tumor growth, progression and immunosuppression in response to some type of cellular or tissue damage.50 They likened the cellular or tissue damage to “the match that lights the fire” and the inflammatory cells and cytokines to “the fuel that feeds the flames”. This may well fit the molecular pathological relationship between the esophageal squamous cell epithelia at risk and infiltrating macrophages into the epithelia. The injury signals from epithelia by carcinogens...
The contribution of macrophages infiltrating into the epithelia as a biological promotor of early esophageal carcinogenesis, a hypothesis. (a) Low-power view of an esophageal squamous cell carcinoma in situ (CIS, left side) and non-neoplastic mucosa (right side) excised by endoscopic submucosal dissection. Nuclear labeling of Ki-67 is prominent in the whole layers of the CIS with the infiltration of numerous CD163- and/or CD204-positive macrophage infiltration. i: Higher-power view of CD204 immunoreaction in the CIS (square, i). ii, Higher power view of CD204 immunoreaction in the non-neoplastic mucosa (square, ii). (b) Macrophages observed in the neoplastic esophageal mucosa may promote the growth and migration of the squamous epithelia by activating p38 MAP kinase and inducing the secretion of IL6. (c) Working hypothesis of the roles of macrophages in early esophageal carcinogenesis. (Parts of the figure were adopted from Hanahan D and Weiberg R. Hallmarks of cancer: the next generation. Cell 2011; 144: 646–74. DOI: 10.1016/j.cell.2011.02.013).
such as acetaldehyde (the ‘match’) may recruit macrophages to the focus where they provide the sustained growth and/or migration signals to the injured epithelia (the ‘fuel’).

With the sustained growth signals from the infiltrated macrophages, the amplified “replication stress” may induce accumulations of genetic alterations in the epithelia sufficient for the self-supply of the growth signals to establish autonomous growth mechanisms. Here, we would like to propose that some macrophages may serve as “biological promoters” in the early stage of esophageal carcinogenesis.

CONCLUSION

The findings presented herein imply that diverse cancer biological phenomena are evoked by the various interactions of cancer cells and stromal cells. Therefore, the morphological presence of a cancer is a pathological tissue assembled from numerous microenvironments as the fields of various CSIs. The understanding of “cancer as a tissue” must be the essential assignment of tumor pathology that will provide cues to approach the diversity and organ specificity of cancer-related biology that may not be answered by the mere knowledge of the alterations of genes and molecules of cancer cells.

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REFERENCES

1 Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57–70.
2 Usami Y, Chiba H, Nakayama F et al. Reduced expression of claudin-7 correlates with invasion and metastasis in squamous cell carcinoma of the esophagus. Human Pathol 2006; 37: 569–77.
3 Ueda J, Semba S, Chiba H et al. Heterogeneous expression of claudin-4 in human colorectal cancer: Decreased claudin-4 expression at the invasive front correlates cancer invasion and metastasis. Pathobiology 2007; 74: 32–41.
4 Matsuda Y, Semba S, Ueda J et al. Gastric and intestinal claudin expression at the invasive front of gastric carcinoma. Cancer Sci 2007; 98: 1014–9.
5 Masuda R, Semba S, Mizuuchi E, Yanagihara K, Yokozaki H. Negative regulation of the tight junction protein tricellulin by snail-induced epithelial-mesenchymal transition in gastric carcinoma cells. Pathobiology 2010; 77: 106–13.
6 Kato H, Semba S, Miskad UA, Seo Y, Kasuga M, Yokozaki H. High expression of PRL-3 promotes cancer cell motility and liver metastasis in human colorectal cancer: A predictive molecular marker of metachronous liver and lung metastases. Clin Cancer Res 2004; 10: 7318–28.
7 Miskad UA, Semba S, Kato H, Yokozaki H. Expression of PRL-3 phosphatase in human gastric carcinomas: Close correlation with invasion and metastasis. Pathobiology 2004; 71: 176–84.
8 Miskad UA, Semba S, Kato H et al. High PRL-3 expression in human gastric cancer is a marker of metastasis and grades of malignancies: An in situ hybridization study. Virchows Arch 2007; 450: 303–10.
9 Matsukawa Y, Semba S, Kato H, Koma Y, Yanagihara K, Yokozaki H. Constitutive suppression of PRL-3 inhibits invasion and proliferation of gastric cancer cell in vitro and in vivo. Pathobiology 2010; 77: 155–62.
Mizuuchi E, Semb S, Kodama Y, Yokozaki H. Down-modulation of keratin 8 phosphorylation levels by PRL-3 contributes to colorectal carcinoma progression. *Int J Cancer* 2009; **124**: 1802–10.

Matsukawa Y, Semb S, Kato H, Ito A, Yanagihara K, Yokozaki H. Expression of the enhancer of zeste homolog 2 is correlated with poor prognosis in human gastric cancer. *Cancer Sci* 2006; **97**: 484–91.

Wang H, Quah SY, Dong JM, Manser E, Tang JP, Zeng Q. PRL-3 down-regulates PTEN expression and signals through PI3K to promote epithelial-mesenchymal transition. *Cancer Res* 2007; **67**: 2922–6.

Battistelli C, Cicchini C, Santangelo L et al. The Snail repressor recruits EZH2 to specific genomic sites through the enrollment of the IncRNA HOTAIR in epithelial-to-mesenchymal transition. *Oncogene* 2017; **36**: 942–55.

Usami Y, Satake S, Nakayama F et al. Snail-associated epithelial-mesenchymal transition promotes osteopagheal squamous cell carcinoma motility and progression. *J Pathol* 2008; **215**: 330–39.

Hanahan D, Coussens LM. Accessories to the crime: Functions of cells recruited to the tumor microenvironment. *Cancer Cell* 2012; **21**: 309–22.

Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. *J Cell Sci* 2012; **125**: 5591–6.

Kalluri R. The biology and function of fibroblasts in cancer. *Nature Rev Cancer* 2016; **16**: 582–98.

Ishii G, Ochiai A, Neri S. Phenotypic and functional heterogeneity of cancer-associated fibroblasts within the tumor microenvironment. *Adv Drug Deliv Rev* 2016; **99**: 186–96.

Tsukada T, McNutt MA, Ross R, Gown AM. HHF35, a muscle actin-specific monoclonal antibody. II. Reactivity in normal, reactive, and neoplastic human tissues. *Am J Pathol* 1987; **127**: 389–402.

Garin-Chesa P, Old LJ, Rettig WJ. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proc Natl Acad Sci U S A* 1990; **87**: 7235–9.

Sugimoto H, Mundel TM, Kieran MW, Kalluri R. Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer Biol Ther* 2006; **5**: 1640–46.

Orima A, Weinberg RA. Heterogeneity of stromal fibroblasts in tumors. *Cancer Biol Ther* 2007; **6**: 618–9.

Ochiai A, Yasui W, Tahara E. Growth-promoting effect of gastrin on human gastric carcinoma cell line TMK-1. *Jpn J Cancer Res (Gann)* 1985; **76**: 1064–71.

Hojo H. Establishment of cultured cell lines of human stomach cancer–origin and their morphological characteristics. *Nigata Igakukai Zasshi* 1977; **91**: 737–52; (in Japanese).

Motoyama T, Hojo H, Watanabe H. Comparison of seven cell lines derived from human gastric carcinomas. *Acta Pathol Jpn* 1986; **36**: 65–83.

Yokozaki H, Ito M, Yasui W et al. Biologic effect of human hepatocyte growth-factor on human gastric-carcinoma cell lines. *Int J Oncol* 1993; **3**: 89–93.

Yokozaki H. Molecular characteristics of eight gastric cancer cell lines established in Japan. *Pathol Int* 2000; **50**: 767–77.

Yanagihara K, Seyama T, Tsumuraya M, Kamada N, Yokoro K. Establishment and characterization of human signet ring cell gastric carcinoma cell lines with amplification of the c-myc oncogene. *Cancer Res* 1991; **51**: 381–6.

Semb S, Kodama Y, Ohnuma K et al. Direct cancer-stromal interaction increases fibroblast proliferation and enhances invasive properties of scirrhous-type gastric carcinoma cells. *Br J Cancer* 2009; **101**: 1365–73.

Yashiro M, Chung YS, Kubo T, Hato F, Sowa M. Differential responses of scirrhous and well-differentiated gastric cancer cells to orthotopic fibroblasts. *Br J Cancer* 1996; **74**: 1096–103.

Nakazawa K, Yashiro M, Hirakawa K. Keratinocyte growth factor produced by gastric fibroblasts specifically stimulates proliferation of cancer cells from scirrhous gastric carcinoma. *Cancer Res* 2003; **63**: 8848–52.

Karnoub AE, Dash AB, Yoo AP et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 2007; **449**: 557–63.

Mishra PJ, Mishra PJ, Humeniuk R et al. Cancer-associated fibroblast-like differentiation of human mesenchymal stem cells. *Cancer Res* 2008; **68**: 4331–9.

Ishii G, Sangai T, Oda T et al. Bone-marrow-derived myofibroblasts contribute to the cancer-induced stromal reaction. *Biochem Biophys Res Commun* 2003; **309**: 232–40.

Ishii G, Ito T-K, Aoyagi K et al. Presence of human circulating progenitor cells for cancer stromal fibroblasts in the blood of lung cancer patients. *Stem Cells* 2007; **25**: 1469–77.

Quante M, Tu SP, Tomita H et al. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell* 2011; **19**: 257–72.

Mori T, Kiyono T, Imabayashi H et al. Combination of hTERT and bmi-1, E6, or E7 induces prolongation of the life span of bone marrow stromal cells from an elderly donor without affecting their neurogenic potential. *Mol Cell Biol* 2005; **25**: 5183–95.

Nishimura K, Semb S, Aoyagi K, Sasaki H, Yokozaki H. Mesenchymal stem cells provide an advantageous tumor microenvironment for the restoration of cancer stem cells. *Pathobiology* 2012; **79**: 290–306.

Kanzawa M, Semb S, Hara S, Itoh T, Yokozaki H. WNT5A is a key regulator of the epithelial-mesenchymal transition and cancer stem cell properties in human gastric carcinoma cells. *Pathobiology* 2013; **80**: 235–44.

Takahashi K, Naito M, Takeyama M. Development and heterogeneity of macrophages and their related cells through their differentiation pathways. *Pathol Int* 1996; **46**: 473–85.

Naito M. Macrophage differentiation and function in health and disease. *Pathol Int* 2008; **58**: 143–55.

Takeyama M, Komohara Y. Role of tumor-associated macrophages in human malignancies: Friend or foe? *Pathol Int* 2016; **66**: 491–505.

Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004; **25**: 677–86.

Sica A, Larghi P, Mancino A et al. Macrophage polarization in tumour progression. *Semin Cancer Biol* 2008; **18**: 349–55.

Virchow R. *Die Krankhaften Geschwülste*. Berlin: Verlag von August Hirschwald 1863.

Pulford KA, Rigney EM, Micklem KJ et al. KP1: A new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. *J Clin Pathol* 1989; **42**: 414–21.

Högger P, Dreier J, Droste A, Buck F, Sorg C. Identification of the integral membrane protein RM3/1 on human monocytes as a glucocorticoid-inducible member of the scavenger receptor cysteine-rich family (CD163). *J Immunol* 1998; **161**: 1883–90.

Tomokiyu R, Jinnouchi K, Honda M et al. Production, characterization, and interspecies reactivities of monoclonal antibodies against human class A macrophage scavenger receptors. *Atherosclerosis* 2002; **161**: 123–32.

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migration and invasion of cancer cells. Oncotarget 2017; 8: 106071–88.
87 Wolpe SD, Davatelis G, Sherry B et al. Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. J Exp Med 1988; 167: 570–81.
88 Wu Y, Li Y-Y, Matsushima K, Baba T, Mukaida N. CCL3-CCR5 axis regulates intratumoral accumulation of leukocytes and fibroblasts and promotes angiogenesis in murine lung metastasis process. J Immunol 2008; 181: 6384–93.
89 Yoshimura T, Yuhki N, Moore SK, Lerman MI, Leonard EJ. Human monocyte chemoattractant protein-1 (MCP-1). Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. FEBS Lett 1989; 244: 487–93.
90 Komohara Y, Takeya M. CAFs and TAMs: Maestros of the tumour microenvironment. J Pathol 2017; 241: 313–5.
91 Herrera M, Herrara A, Dominguez G et al. Cancer-associated fibroblast and M2 macrophage markers together predict outcome in colorectal cancer patients. Cancer Sci 2013; 104: 437–44.
92 Hashimoto O, Yoshida M, Koma Y-I et al. Collaboration of cancer-associated fibroblasts and tumour-associated macrophages for neuroblastoma development. J Pathol 2016; 240: 211–23.
93 Kumagai Y, Sobajima J, Higashi M et al. Tumor-associated macrophages and angiogenesis in early stage esophageal squamous cell carcinoma. Esophagus 2016; 13: 245–53.