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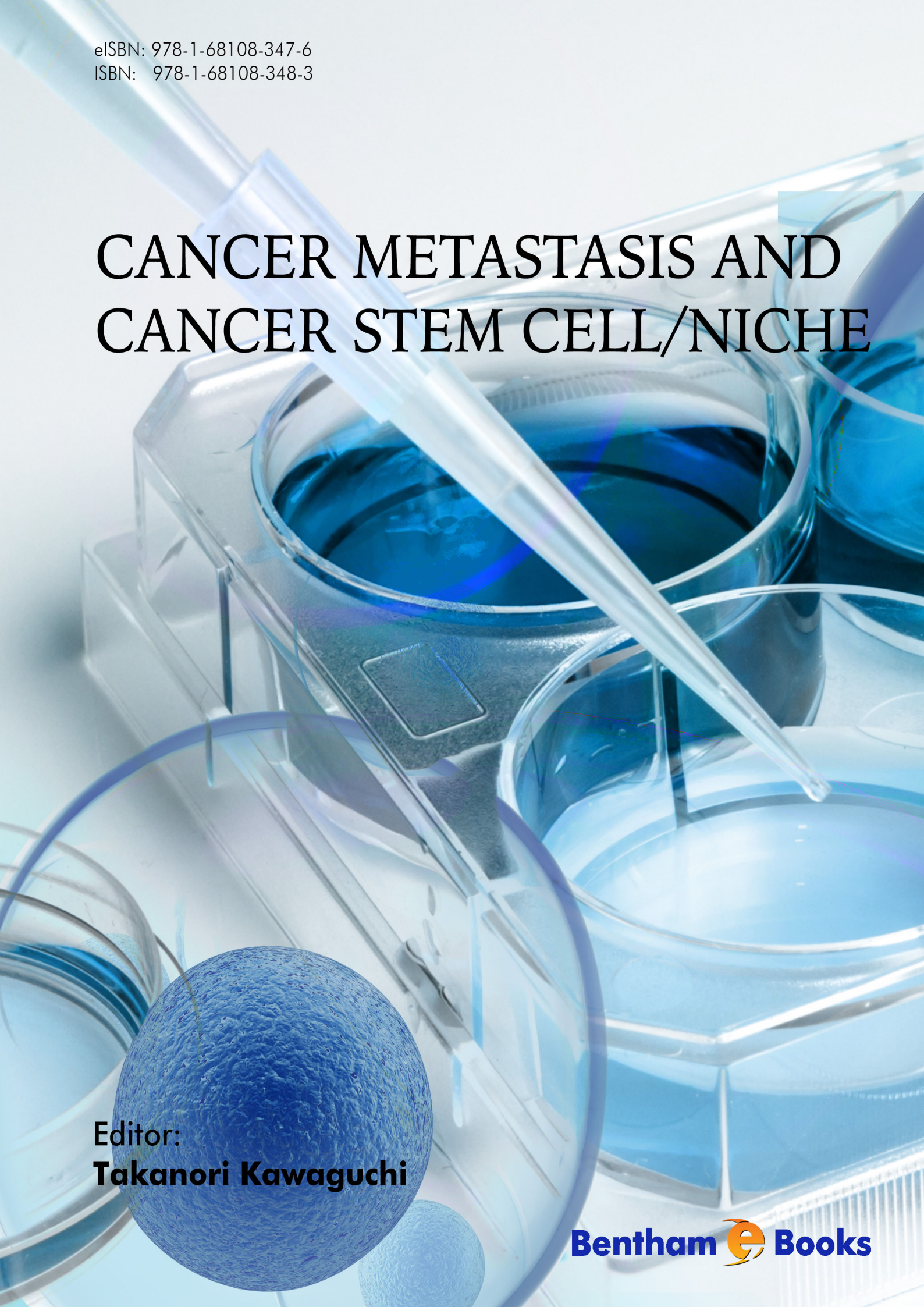
CANCER METASTASIS AND CANCER STEM CELL/NICHE



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Cancer Metastasis and Cancer Stem Cell/Niche

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CONTENTS

FOREWORD

Our knowledge of cancer progression has grown exponentially since the discovery of oncogenes and our ability to model carcinogenesis and cancer initiation. Still the major challenge faced by physicians is the prevention and treatment of metastasis, the main reason for cancer related deaths. Our understanding of metastasis has lagged behind that of primary tumor biology and even with a surge in metastasis research we are far behind grasping its complexities.

Progress has been hampered by the research paradigm itself, which states that metastasis is an extremely late event in micro-evolutionary and temporal scales and driven by a tumor centric view. This information was mostly derived from models that use highly genetically aberrant and aggressive cancer cell line models and ignored the tumor microenvironment in the target organs. Thus, the field has accepted that metastases are an aggressive variant of the primary tumor and that these lesions share most characteristics that are mostly autonomous. This conclusion supported that primary tumor information should be sufficient to target the metastasis. So far this paradigm has shown moderate success at best in revealing the intricacies of metastasis and very little in yielding successful therapies to stop metastasis.

This book on metastasis is timely as we are at a turning point in metastasis research, which will impact the future of patient's treatment. The focus of this book is key to highlight how little we understand about cancer metastasis but also shows how great progress has been made in understanding the complexities of metastasis. This includes accepting that the complexities of metastasis biology are different from the primary tumor and that the target organ microenvironments play key roles in defining the biology of the metastatic cancer cells.

The chapters in this book are centered around the idea that there are minor populations in primary tumors termed cancer stem cells or cancer initiating cells that appear to also have the power to fuel metastasis. The chapters cover the role of the microenvironment composed by stromal cells and immune cells in determining the fate of the primary tumor and metastasis progression. The role of pre-metastatic microenvironments is also highlighted and the cancer stem cell concept is integrated with how niche signals impact epigenetic mechanisms that affect the expression of surface markers such as glycans to regulate the CSC behavior. The authors also cover the areas of secreted vesicles as important signaling mediators between the tumor and the stroma and the role of angiogenesis, which is a clear factor for metastatic maintenances. One chapter is focused on the development of imaging modalities to improve the detection of rare cell subpopulations in tumor lesions, and such advancements are critically needed. This becomes critical when thinking that after surgery and treatment and before metastatic outgrowth there is a period of minimal residual disease (MRD). This periods

can last for decades when the seeds of metastasis are dormant and for which we have no evidence-based treatment and imaging modalities. Unfortunately this stems primarily from the lack of our understanding of the biology that defines MRD, dormancy and reactivation. However, the chapters will illustrate that the field has moved forward significantly and new findings are routinely published that are expanding our understanding of metastasis and how to target it.

As mentioned before this book comes at a time when a change is needed if we are going to be successful in targeting metastasis and that requires deepening our understanding of the biology of disseminated disease, developing markers to detect it, image it and come up with new therapeutic strategies that are based on the direct knowledge of MRD biology and metastasis and not inferred from extrapolations based on primary tumor information, that is sometimes gathered a decade or more before manifestation of the metastasis and thus may be a very different disease. The work highlighted in this book is a testament of the excellent work being developed worldwide to target this deadly step of the disease and possibly our best short-term chance to change the course of cancer treatment and improve patient's life.

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PREFACE

A malignant tumor is an actively growing tissue, composed of cells derived from a single cell that has undergone abnormal irreversible differentiation. These cells have two fundamental qualities: invasion and metastasis that induce the risk of cancer. Interestingly, recent researchers suggest that a malignant tumor is originated from cancer stem cells (CSC) accompanied with the niches. In this eBook, I would like to ask how the invasion and metastasis of cancer cells are explained by CSC theory and/or niche theory. The major points are as follows.

1. The fundamental aspects of cancer:
    1. The first point of discussion is about the CSC/ niche in carcinoma *in situ* lesion which appears in various types of cancer including breast and bladder cancers. Where is the niche corresponding to CSC in the above situation and what is the role of the niche in cancer progression?
    2. Cancer metastasis involves two characteristic occurrences: “organ preference metastasis” and “metastatic potential”. Can these metastatic characteristics be explained by CSC /niche theory well?
2. Metastatic phenotypes:
    The mechanisms of cancer metastasis include non-invasiveness (carcinoma *in situ*), lateral/side invasion, stromal invasion, intravasation, circulation, arrest, attachment, extravasation, early growth, growth, and sometimes secondary/tertiary metastasis. This is followed by cellular/molecular mechanisms including cellular adhesion molecules such as carbohydrates, chemotactic activity by producing pseudopodia and invadopodia, deformability/plasticity and cytostreaming of metastatic cancer cells, and survival of metastatic cancer cells in floating (substrate-independent condition). Can these phenotypes of metastatic cancer cells be explained by cancer stem cell theory?
3. The goal of this study is clinical application of the knowledge obtained by CSC/niche theory. CSC/niche targets have important therapeutic implications. The role of CSC/ niche on therapies for the prevention, maintenance therapy, personalized care, and perhaps even integrative care of cancer will be discussed.

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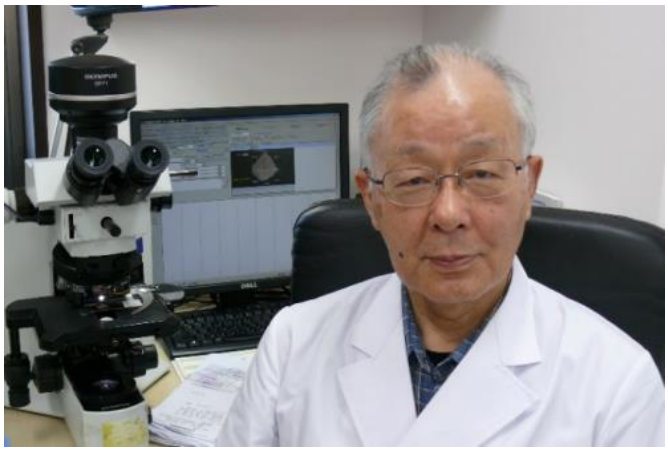
Biography

My research on cancer metastasis began in 1968, at the 2nd Department of Pathology, Fukushima Medical University College of Medicine (Professor and Chairman, Kyuya Nakamura) and continued till 2006. Since 2006, I have been working at the Division of Nursing (Professor and Chairman, Takashi Honda) and the Department of Pathology, Aizu Chuo Hospital (Head, Takanori Kawaguchi). During this period, I collaborated with many researchers. In 1980, I visited the Department of Tumor Biology, MD Anderson Cancer Center and Tumor Institute in Houston (Professor and Chairman, Garth L. Nicolson), where I studied the metastasis of B16 melanoma variant sublines for a year. During the past 50 years, there have been great advances in metastasis research, for example, electron microscopy revealed cellular/subcellular behavior of tumor cells in metastasis. Molecular biology facilitated identification of the molecules involved in metastasis. For the last 100 years, the biggest issue associated with cancer metastasis research was a conflict of "anatomical-mechanical theory" and "seed and soil theory." However, our research indicated that the anatomical–mechanical theory can be replaced by tissue injury hypothesis (microinjury hypothesis), and this concept was supported by the large scale of research on human cancer. Thus, I believe that metastasis occurs in the affinity of tumor cells and soil (niche), where soil causes the tumor cells to become immature.



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CHAPTER 1

Fundamental Relationships Between Cancer Stem Cells, the Cancer Stem Cell Niche and Metastasis

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Abstract: Parallels drawn between stem cells and cancer are not new. However, these shared features are becoming increasingly important as our understanding of disseminated, recurrent and metastatic cancer cell biology continues to develop. Indeed, nearly all cancer-related deaths are the result of recurrent and metastatic disease, highlighting the need for a more comprehensive schema of how tumors colonize new sites, resist therapy and evolve. In this chapter, we compare the phenotypes of stem cells, cancer stem cells (CSCs) and metastatic cells, highlighting notable points of contrast. We begin with an introduction to stem cell biology, tumor-initiating CSCs, metastatic cells and discuss shared features. The implication of the stem-like phenotype extends to many characteristics of cell biology: cell division, differentiation, morphology, gene expression, motility, invasion, clonogenicity, capacity for colonization, metabolism and the interaction of these cells with their surrounding microenvironment. Stem cell phenotypes are highly complex and, while there may be a number of shared features, there are important elements that are uniquely tissue-dependent. While staunch definitions based upon a single biomarker of stemness have proven inadequate in broader applications, seeing universal themes of the stem cell phenotype will provide critical insights for studying cancer. Our understanding of this complex biology is critical for developing rational and dynamic therapeutic interventions for patients with recurrent and metastatic cancer.

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Keywords: Biomarker, Cancer stem cell, Cancer stem cell niche, Clonogenicity, Dissemination, Dormancy, Epithelial-mesenchymal transition, Heterogeneity, Metabolism, Metastasis, Metastasis-initiating cells, Metastatic cell, Metastatic colonization, Metastatic niche, Pluripotency, Quiescence, Review, Self-renewal, Stem cell, Stem cell niche, Tumor progression, Tumorigenesis, Tumorigenic cells, Tumor-initiating cell.

INTRODUCTION

The observation that features of a cancer cell resemble developmentally primitive cells dates back over 150 years [1]. This has proven to be a perspicacious observation indeed, given the similarities between cancer cells and stem cells with respect to their dynamic regulation of mitosis, gene expression, migration, metabolism and self-sustainability. Cell division is a critical component of carcinogenesis and early observations prompted the notion that cancer may arise from a stem cell [2, 3]. Additionally, the therapeutic significance of cancer cells displaying a stem cell-like phenotype has been more recently appreciated after patients whose cancer was treated with chemotherapy and were without evidence of disease, experienced recurrence of disease months or years later. These observations suggested that there was perhaps a population of mitotically quiescent cancer cells unaffected by chemotherapy that survived, proliferated and gave rise to the recurrent tumor or metastatic disease [4]. Tumor-initiating cells are the proposed "cancer stem cells" (CSCs). Given the importance of these phenotypes in the control of cancer, here we compare the shared and distinct biological features of normal stem cells, CSCs and metastatic cancer cells and consider the therapeutic significance of these insights.

FEATURES OF STEMNESS

Stem Cells in Normal Physiology and Development

Stem cells are populations of cells with the capacity to divide symmetrically, where the resulting daughter cells retain an equal potential to produce cells of a given lineage, or asymmetrically, resulting in more differentiated daughter cells that are narrowed in the variety of cells in that lineage they can produce [5]. *In vivo,* it was thought that stem cells spend the majority of their cell cycle in

mitotic quiescence [6], and upon stimulation by tissue damage or exogenous factors they can be induced to divide. More recently, in addition to quiescent stem cells, it has been shown that a population of $Lgr5^+$stem cells also undergo active proliferation [7]. Daughter cells resulting from asymmetric divisions may become the more actively proliferating yet shorter-lived transit-amplifying cells which serve to regenerate tissue when required [8]. When stem cells are cultured *in vitro* however, conventional passaging methods may select for rapidly proliferating cells [9], explaining in part the dissonance between these two phenotypes. Ultimately, the orchestrated proliferation and differentiation of normal stem cells serve to reconstitute tissue lost to damage, aging and use. In a similar way, tumor cells remaining after selection by chemotherapy would be the "stem cells" of that tumor. These CSCs could eventually be stimulated to divide, resulting in the maintenance of slow-cycling CSCs. Stochastic changes in other daughter cells would re-establish tumor heterogeneity and a population of rapidly dividing cancer cells responsible for recurrence of disease.

The Cancer Stem Cell Phenotype

Morphology, Differentiation and Self Renewal

Discussion of CSCs envelops many concepts related to the origin of malignant cells, how selective processes change the tumor cell population over time (*i.e.* tumor progression), the ability of cells to successfully colonize new sites (*i.e.* metastasis), and the features of therapy-resistant cancer cells in disease recurrence (summarized in Table **1** and Fig. **1**). Although a so-called "tumor initiating cell" may very well fit many of the aforementioned criteria, this nomenclature describes many of the later aspects of CSC biology without reference necessarily to their origin, with which we begin our discussion. Morphologic observations of cancers and embryonic tissues gave rise to the first ideas connecting cancer with stem cells. The strikingly heterogeneous composition of teratomas, containing teeth, hair, and sundry embryonic tissues led to the hypothesis that these tumors may come from a stem cell [10]. Further studies extended this observation and demonstrated the stemness of teratoma cells by showing their potential to differentiate into a multitude of tissues [11]. Beyond potency, normal stem cells, tumorigenic cancer cells and metastatic cells must all have the capacity for

CHAPTER 2

Regulation of Cell Surface Glycan Expression in Cancer Stem Cells

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Abstract: Cell surface glycans are recognized to be good markers for human pluripotent embryonic stem cells. Typical glycan markers for human embryonic stem cells include SSEA-3, SSEA-4, TRA-1-60 and SSEA-5. Some of these glycans are recently known to be frequently expressed in human cancers, especially in cancer stem cells. Cell surface glycans undergo drastic changes also during malignant transformation, and the glycans which preferentially appear in cancers are clinically utilized as diagnostic markers for human cancers. Such tumor marker glycans include sialyl Lewis A and sialyl Lewis X, expression of which we recently showed to be enhanced in cancer stem-like cells that had undergone epithelial-mesenchymal transition. Sialyl Lewis A was also shown to be expressed in human embryonic stem cells, and to behave as an embryonic stem cell specific marker. Thus, a glycan initially described as a cancer-associated glycan in the cancer research field is now known to be an embryonic stem cell marker, while glycans formerly regarded to be typical embryonic stem cell markers in the embryology field are now shown to be cancer stem cell markers. This suggests the presence of a common induction mechanism for these glycans shared by embryonic stem cells and cancer stem cells. However, the regulatory mechanisms for stem-cell specific glycan expression remain largely unknown. In this chapter we will introduce how glycan-related genes responsible for synthesis of the stem-cell specific glycans are regulated through specific epigenetic modification, by niche-associated microenvironmental factors such as hypoxia, and during a morphogenic process like epithelial-mesenchymal transition.

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Keywords: Cancer stem cells, DNA methylation, Dupan-2, Embryonic stem (ES) cells, Epigenetic silencing, Epithelial-mesenchymal transition (EMT), Histone deacetylation, Histone methylation, Hypoxia inducible factor, Selectins, Sialyl Lewis A, Sialyl Lewis X, Siglecs, SLC26A2, SSEA-3, SSEA-4, SSEA-5, ST6GalNAc6, Sulfate transporter, TRA-1-60.

INTRODUCTION

Perhaps the most important premise assumed in the theory of cancer stem cells is that they are derived from normal stem cells in the given tissue where the process of carcinogenesis takes place. If this is true, the cancer cells may inherit remnants of epigenetic patterns of gene expression from the normal stem cells through the epigenetic memory, and may share with them the common microenvironments which are termed as niche *in toto*, and will actively undergo extensive morphologic changes such as EMT/MET required for "organogenesis," which may be represented as "metastasis" in the case of cancer cells. Of course the behavior of cancer stem cells will not be exactly the same as normal stem cells, as the cancer stem cells will exhibit novel features that will be acquired through the reprogramming process during the course of carcinogenesis. It is conceivable that although cancer initiating cells originate from normal tissue stem cells in the given organs, there is still not solid evidence indicating that the origin of cancer initiating cells is strictly confined to normal stem cells in every tissue and organ. Therefore, the terms "tumor-initiating cell," "tumor-propagating cells" or "cancer stem-like cell" are sometimes recommended instead of cancer stem cell. There is, however, no doubt that the concept of cancer stem cells has significantly contributed to our understanding of many biological aspects of cancers.

CANCER STEM CELLS AND EPIGENETIC SILENCING OF GLYCOGENES

Induction of Typical Cancer-associated Glycans Through Epigenetic Silencing of Glycan-related Genes Responsible for Synthesis of Normal Glycans

It has long been known that glycans undergo drastic changes upon carcinogenesis. The glycans preferentially appear in cancer cells, such as sialyl Lewis A and sialyl

Lewis X, are utilized in clinical diagnosis of cancers. We recently showed that expression of these cancer-associated glycans is induced in cancer cells at the early stages of carcinogenesis through epigenetic silencing of several glycan-related genes responsible for synthesis of normal glycans.

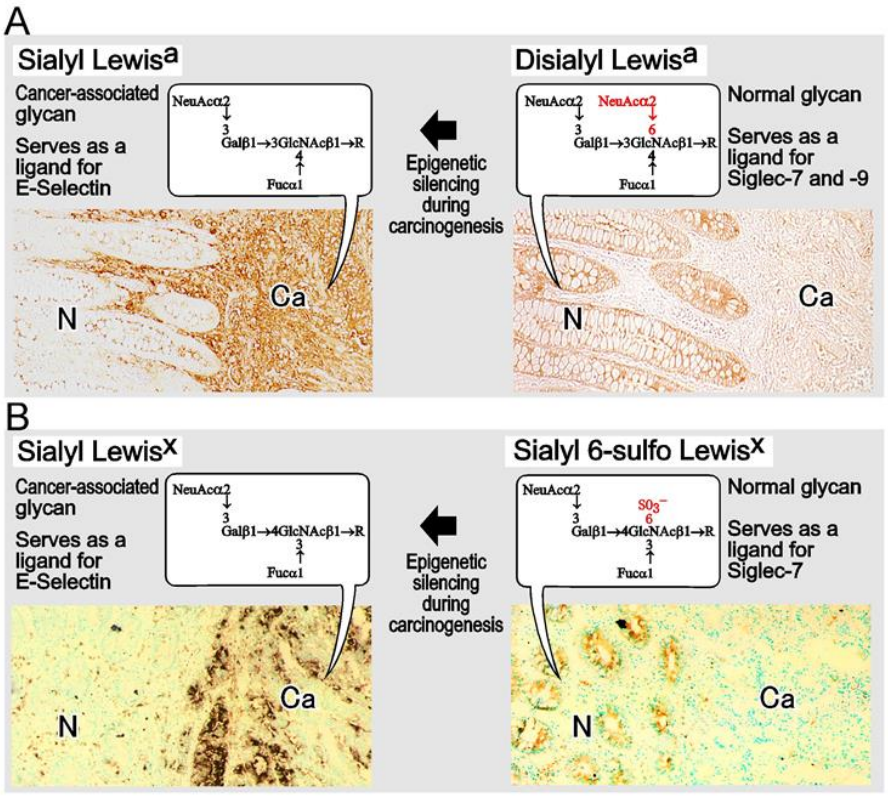

Fig. (1). Examples of interconversion of normal glycans into cancer-associated glycans. Panel **A**, transition of normal glycan, disialyl Lewis A, to cancer-associated glycan, sialyl Lewis A, upon malignant transformation. Panel **B**, conversion of normal glycan, sialyl 6-sulfo Lewis X to a cancer-associated glycan, sialyl Lewis X glycan upon malignant transformation. Typical distribution patterns shown were obtained by immunohistochemical staining using specific anti-glycan antibodies of consecutive sections prepared from colon cancer tissues. Ca, cancer cells; N, non-malignant epithelial cells. (Adapted from references [5 - 7]).

A variety of glycans are expressed in normal epithelial cells, expression of some of which is conventional in that they are also constitutively expressed in cancers. In contrast, some other normal glycans exhibit preferential expression in non-malignant epithelial cells, and tend to decrease or disappear and be replaced by cancer-associated glycans upon malignant transformation. Such normal glycans

CHAPTER 3

Tumor Endothelial Cells and Cancer Progression

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Abstract: Tumor growth and metastasis are facilitated by the formation of new blood vessels, a process known as angiogenesis. The blood vessels formed around the tumor supply it with oxygen and nutrients, which together support its progression. Moreover, the newly formed blood vessels serve as channels through which tumor cells metastasize to distant organs. Tumor blood vessels, and especially the endothelial cells lining tumor blood vessels (tumor endothelial cells, TECs), have therefore gained interest as targets in cancer therapy. Although newly formed tumor blood vessels originate from pre-existing, normal vessels, they have a distinctively abnormal phenotype, including important morphological alterations. The balance between the angiogenic stimulators and inhibitors regulates angiogenesis in the tumor microenvironment. Furthermore, TECs constitute a heterogeneous population, exhibiting characteristics induced largely by tumor microenvironmental factors. In this chapter we review recent studies on TEC abnormalities regarding to cancer progression and consider the therapeutic implications thereof.

Keywords: Angiogenesis, Angiogenic factor, Anti-angiogenic therapy, Basement membrane, Blood vessel, Cancer, Drug resistance, Endothelial cell, Heterogeneity, Hypoxia, Invasion, Metastasis, Migration, Pericyte, Side effect, Tumor, Tumor angiogenesis, VEGF.

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Takanori Kawaguchi (Ed.)

TUMOR ANGIOGENESIS

Angiogenesis leads to the formation of new blood vessels and is essential for tumor progression. Tumor blood vessels supply the tumor with needed oxygen and nutrients, in addition to removing waste products from tumor tissue. However, tumor vessels also provide a route to metastasis [1, 2]. The endothelial cells that line tumor blood vessels (TECs) have emerged as important targets of angiogenic inhibitors (anti-angiogenic therapy) and provide a strategy for cancer treatment, with many anti-angiogenic drugs developed and tested to date [3]. The basis for pursuing this therapy can be summarized as follows: i) The survival of a large population of tumor cells depends on a few TECs, such that targeting TECs may be more efficient than targeting tumor cells. ii) Since TECs exhibit similar characteristics regardless of their tumor of origin, a single, effective anti-angiogenic drug could be used to treat many forms of cancer. iii) It was thought that TECs are genetically stable unlike tumor cells, and therefore do not become drug resistant. However, recent studies have shown that TECs from primary tumor sites differ from normal endothelial cells (NECs) and their makeup in various tumor types is heterogeneous. Moreover, while anti-angiogenic drugs were thought to be less toxic than other cytotoxic drugs, it is now clear that they may induce severe side effects, such as lethal hemoptysis [4, 5] and intestinal perforation [6, 7]. Accordingly, an important goal in cancer therapy is to develop novel and safer tumor anti-angiogenic agents, which in turn depends on a thorough understanding of the biology of TECs.

TUMOR BLOOD VESSELS ARE MORPHOLOGICALLY ABNORMAL

Tumor blood vessels differ in many ways from normal blood vessels (Fig. **1a**). Specifically, tumor vessels are not organized in the same hierarchal branching pattern (*i.e.*, from arteries to capillaries and then veins) as the normal vasculature [8]. Their underlying basement membranes are of varying thicknesses, and their TECs do not form regular monolayers [9], unlike in normal blood vessels [10]. Although pericytes are present, they form abnormally loose association with TECs [11]. Consequently, tumor blood vessels are leaky. Other abnormalities of tumor blood vessels have been attributed to the unbalanced expression of angiogenic factors and inhibitors (Fig. **1b**). In addition to the above

characteristics, tumor blood vessels are often immature morphologically, The high interstitial fluid pressure in cancer causes vessel collapse and blood flow is compromised. Furthermore, tumor vessels show chaotic blood flow and they are leaky because of the loose interconnections in endothelium [12]. These features of tumor vasculature may be one of reason why cancers are usually hypoxia even though they are highly vascularized. Hypoxia in cancer is the cause of resistance to radiation therapy [13].

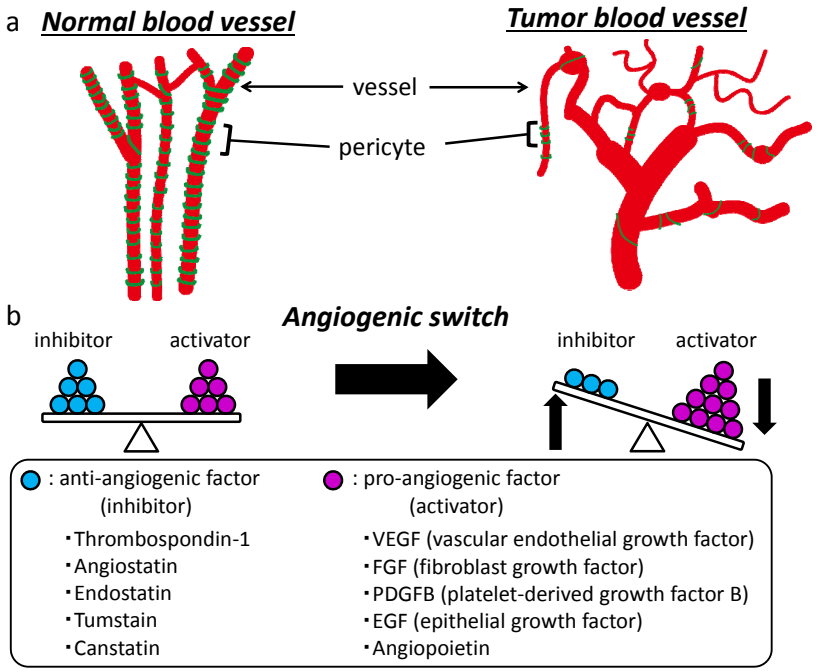

Fig. (1). Differences in the blood vessels of tumors and normal tissue.
(a) Tumor vessels show excessive branching but lack the arteriole-capillary-venule hierarchy. As a result, blood flow through these vessels is chaotic. Resident pericytes in tumors associate with endothelial cells loosely. **(b)** Tumors are characterized by imbalances between the levels of pro-angiogenic (activators) and anti-angiogenic (inhibitors) factors. The up-regulation of activators *vs.* inhibitors causes an angiogenic switch in the tumors.

TECs are morphologically irregular, with long cytoplasmic projections extending across the lumen, whereas NEC are uniform. The endothelial gaps and transcellular fenestrae in the walls of tumor blood vessels result in hemorrhage and plasma leakage, two common properties of tumors. Moreover, they allow filling in by adjacent tumor cells and provide a mechanism for tumor cell

CHAPTER 4

Metastatic Cancer Stem Cell Niche and the Toll-like Receptor 4 (TLR4)-mediated Premetastatic Microenvironment

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Abstract: Cancer metastasis is one of the most crucial problems in the field of medical research. There are two types of approaches to find a new way to treat cancer metastasis; Targeting cancer stem cells, or stromal cells in the premetastatic phase. Interactions between these two elements synergistically enhance the survival of metastatic cancer cells and promote their re-growth in the distant organ. In this review, first we summarize the recent trends in cancer stem cell studies. Then, we discuss the premetastatic phase, based on our investigations. We also review various types of tumor-associated stromal cells (monocyte/macrophage, fibroblast, adipocyte, dendritic cell, neutrophil, and natural killer cell) in relations with the tumor microenvironment formation. Moreover, dormant tumor cells and circulating tumor cells are included in this review.

Keywords: Biglycan, CCL2, CCR2, Circulating tumor cell, Dormant cell, EMT, Eph, Ephrin A1, HMGB1, Inflammation, MD-2, Metalloprotease, Organ tropism, Premetastatic microenvironment, S100A8, SAA3, TGF-β, TLR4, TNFα, VEGF.

I. OVERVIEW

I-a. Outline of Tumor Metastasis

It is often said that tumor metastasis do not just happen. Actually, it is a sequence

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Takanori Kawaguchi (Ed.)

of events including dissemination of tumor cells from (a) primary tumor formation, (b) local invasion, (c) intravasation, (d) cell survival during circulation in blood vessels, (e) extravasation, (f) settlement of tumor cells at distant organ, and (g) metastatic tumor regrowth. Every single step has been intensively scrutinized so that myriads of reports are available. The outline of "the premetastatic phase"/metastasis is shown in Fig. (**1**).

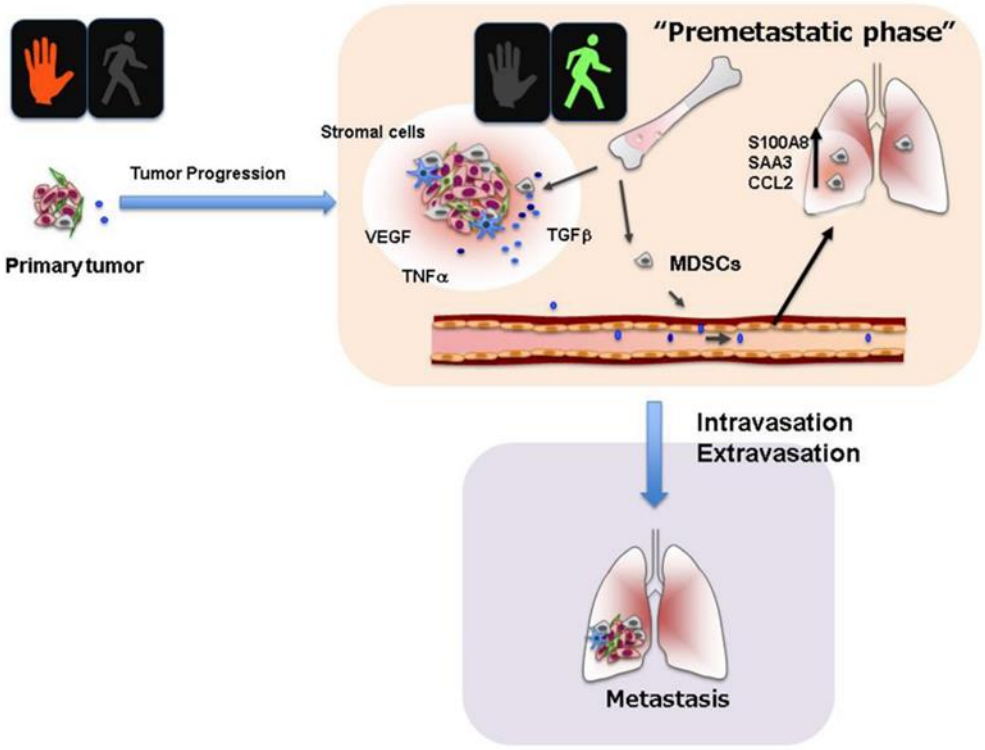

Fig. (1). Outline of the pre-metastatic phase.
The recruitment of myeloid-derived cells is a crucial step in metastasis. In the early phase of the primary tumor, it is not ready for myeloid-derived cells (monocytes, MDSCs) to recruit primary tumor sites and distant organs (in this case, lung; Don't walk). Once tumors develop certain size, the primary tumor microenvironment secretes cytokines/chemokines (*i.e.* VEGF, TNFα, and TGF-β) to supply to myeloid-derived cells. These cytokines can send a signal to myeloid-derived cells in bone marrow as "Walk".

Interestingly, different molecule comes forward with every single step toward metastasis. The data that knockdown of individual molecular, any given molecule

highlighted in the research, by using knockout mouse or shRNA techniques seriously did hurt macroscopic metastasis indicate that tumor metastasis is a balance as subtle as on the edge of a cliff. In other words, since many molecules should be orchestrated for cancer metastasis, just disordered one piece is enough to make it incomplete.

For instance, cathepsins were indispensable to local tumor invasion [1, 2]. These metalloproteases were generated by macrophages upon stimulation of IL-4, secreted by tumor cells or T cells nearby [1, 3]. During intravasation, key molecules between tumor cells and macrophages became epidermal growth factor (EGF) and colony stimulating factor-1 (CSF-1) instead [4]. Selectins prolonged the lifetime of tumor cells in the blood vessels [5]. Metadherin [6] and angiopoietin-like 4 (Angptl4 [7],) were necessary for tumor cells to extravasate in a distant organ. In order to commence the colonization, immigrated tumor cells required matrix metalloproteinase-9 (MMP-9) and stromal cell derived factor-1 (SDF-1) released from stromal cells [8]. Osteopontin from bone marrow derived cells were needed for further growth [9]. Vascular cell adhesion molecule-1 (VCAM-1) is one of the most hopeful therapeutic target molecules. VCAM-1 expressed on the metastatic tumor cells bound integrin α4β1 on tumor-associated macrophages. With the ligand binding, the PI3K-Akt signaling pathway was activated, which caused dissemination of tumor cells in lungs [10, 11]. Again, these proteins are just a small portion. Different types of tumor cells may ask different kinds of molecular sets to metastasize, implying that custom-made regimens should be designed to prevent metastasis. Thus, this fact makes tumor treatment more painstaking.

Recent studies continue to shed light on potential therapeutic target molecules. Retinoic acid receptor responder protein 3 (RARRES3) was found out to be a metastasis suppressor. Phospholipase A1/A2 activity of this protein played an important role to keep tumor cells from initiating metastasis in the lungs [12]. Repression of thrombospondin-1, anti-angiogenic factor, was essential for tumor growth. Interestingly, an investigation uncovered that regulation of thrombospondin-1 in fibroblast was distinct from that in epithelial cells [13]. Cathepsin S promoted brain metastasis by cleaving junctional adhesion molecule-B (JAM-B) to facilitate tumor cells passing through the blood brain barrier [14]. A

CHAPTER 5

Cancer Stem Cell and Clinical Cancer Metastasis in Surgical Oncology

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Abstract: The cancer stem cell (CSC) theory has emerged as an attractive hypothesis for tumor development and progression including metastasis. The theory suggests that tumors consist of subsets of cells with functional heterogeneity in which one small subset has the characteristics of stem cells. These stem cells have the capacity of both self-renewal and heterogeneous differentiation into cancer cells that comprise the tumor. They can also play an important role in invasion, metastasis and, finally, recurrence. Based on the pathgenesis of the cancer metastasis, the recurrences after curative surgery probably develop from the proliferation of occult micro-metastases already established at the time of surgery. The attractive ideas about CSCs hypothesis in metastasis can partially explain the concept of minimal residual disease like occult micro-metastases after curative resections. CSC hypothesis in clinical metastasis is now giving a deep impact on surgical oncology. Efforts to develop diagnostic and therapeutic approach with the successful results from CSC studies would lead to impressive improvement for cancer patients in surgery.

Keywords: Adjuvant chemotherapy, Biomarker, Cancer, Cancer stem cell (CSC), Circulating tumor cells (CTC), Disseminating tumor cells (DTC), Epithelial-mesencymal transition (EMT), Mesenchymal-epithelial transition (MET), Metastasis, Micro-metastases, Prognosis, Recurrence, R0 resection, Surgery, Surgical oncology, Target therapy.

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INTRODUCTION

Cancer metastasis consists of a multistage process during which cancer cells spread from the primary to distant organs [1]. At first, cancer cells detach from the extracellular matrix and invade the surrounding tissue. Cancer cells migrate toward a vascular blood supply by localized proteolysis at the tumor cell-basement membrane interface and then, penetrate thin-walled vessels to gain access to the systemic circulation (intravasation). Cancer cells circulate as individual cells or clusters with some blood components like platelets and arrest in the distant microvascular beds by passive or active mechanisms. Cancer cells migrate into arrested remote organ through the endothelial cells (extravasation) by multiple mechanisms. Adherent cancer cells may migrate across intracellular junctions between adjacent endothelial cells (paracellular route) or they may penetrate through the body of a single endothelial cell (transcellular route). After gaining access to the underlying tissue of arrested remote organ, cancer cells establish reciprocal signaling networks with surrounding stromal cells to promote their growth including neo-vascularization and finally establish metastatic focus. To survive through the multistage process, cancer cells should possess the ability to escape from physical pressures in the vascular stream, anoikis, apoptosis, and the host's immunologic defenses. Although the rate of cancer cell release in cancer patients is unknown, metastasis is regarded as a highly inefficient process in that less than 0.01% of circulating cancer cells eventually succeeds in forming secondary tumor growth by experimental model [2]. This is a result of the elimination of circulating tumor cells that fail to complete all steps in the metastatic process. The metastatic process is realized to be inefficient [3]. Therefore, it has been debatable whether a few cancer cells with fortunate survival and growth can develop the metastasis or whether a unique subpopulation of cancer cells with selective properties for growth and survival can develop the metastasis [4]. However, a lot of findings based on animal models and clinical studies suggest that human tumors are biologically heterogeneous [5] and that the process of clinical metastasis is selective [6].

Recently, the cancer stem cell (CSC) theory has emerged as an attractive hypothesis for tumor development and progression including metastasis [7, 8]. The theory suggests that tumors consist of subsets of cells with functional

heterogeneity in which one small subset has the characteristics of stem cells. These stem cells have the capacity of both self-renewal and heterogeneous differentiation into cancer cells that comprise the tumor [7]. They can also play an important role in invasion, metastasis and, finally, recurrence. Based on the recent findings, CSCs can induce cancer metastasis through multiple pathways and participate in angiogenesis directly and indirectly [9 - 11]. Furthermore, the migrating cancer stem cell concept [12] proposed that CSCs *in situ* can transform to migrating cancer stem cells by epithelial-mesencymal transition (EMT). Then, they disseminate and form metastatic foci. Indeed, cells possessing both the stem and tumorigenic phenotypes of CSCs were derived from human mammary epithelial cells [13].

In this review, impact of CSCs in clinical metastasis is overviewed from surgical oncologist's aspects.

CLINICAL SIGNIFICANCE OF METASTASIS IN SURGERY

Despite the recent advances in diagnostic techniques for early detection of cancer and the progress in therapeutic procedures including surgical resection, chemotherapy, and radiation therapy, prognosis of the patients with cancers is still unsatisfactory. Major problems for the cancer treatment are not the primary tumors but the formation of metastases [14]. Recurrence is the most critical situation in the treatment of cancer patients after curative surgery. Almost all of cancer recurrences are due to metastasis on the distant organs or regional lymph nodes if surgical resection is undergone in curative intent. Clinically, patients with distant metastasis are hardly appropriate to surgical resection which possibly offers the patients with solid cancer an opportunity to live longer and cure. Even though patients underwent curative resection (surgical macroscopically zero residual: R0) resections at the surgery, a significant number of the patients have recurrence with developing systemic metastasis at distant organ within a few years after surgery. Main reason of cancer death remains to be recurrence due to the metastasis. Based on the pathogenesis of the cancer metastasis, these recurrences probably develop from the proliferation of occult micro-metastases already established at the time of surgery [15, 16]. Clinical metastasis, however, detected after surgery varies from patient to patient. Multiple liver metastases

SUBJECT INDEX

F

G

M

Q

R

S

T