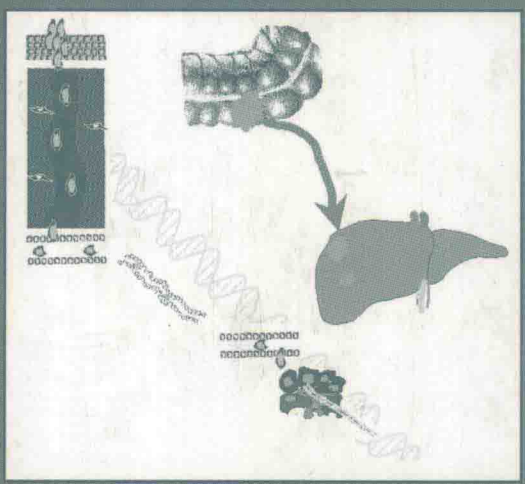
CANCER METASTASIS – BIOLOGY AND TREATMENT

Cancer Metastasis – Related Genes

Edited by

Danny R. Welch



Kluwer Academic Publishers

Cancer Metastasis – Related Genes

Edited by

Danny R. Welch
Jake Gittlen Cancer Research Institute,
The Pennsylvania State University College of Medicine,
Pennsylvania, The United States of America

KLUWER ACADEMIC PUBLISHERS
DORDRECHT / BOSTON / LONDON

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN 1-4020-0522-9

Published by Kluwer Academic Publishers,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

Sold and distributed in North, Central and South America
by Kluwer Academic Publishers,
101 Philip Drive, Norwell, MA 02061, U.S.A.

In all other countries, sold and distributed
by Kluwer Academic Publishers,
P.O. Box 322, 3300 AH Dordrecht, The Netherlands.

Printed on acid-free paper

Printed in the Netherlands.

Cancer Metastasis – Related Genes

Cancer Metastasis – Biology and Treatment

VOLUME 3

Titles published in this Series are:

Volume 1: Cancer Metastasis, Molecular and Cellular Mechanisms and Clinical Intervention.

Editors: Wen G. Jiang and Robert E. Mansel.
ISBN 0-7923-6395-7

Volume 2: Growth Factors and Receptors in Cancer Metastasis.

Editors: Wen G. Jiang, Kunio Matsumoto, and Toshikazu Nakamura.
ISBN 0-7923-7141-0

PREFACE

Being diagnosed with cancer is devastating. But when the cancer cells have to spread to form secondary colonies, the prognosis for the patient is worse. If meaningful improvements in survival are to occur, then control of metastasis will be a foundation. Relatively little is known about the control of the metastatic process at the molecular level. This volume begins to explore our current knowledge regarding the underlying molecular and biochemical mechanisms controlling the metastatic phenotype. While all of the authors attempted to put their findings into a context for translation to the clinical situation, the state-of-the-art does not fully allow this. Nonetheless, we write these summaries of our work as an early effort toward that end. I am grateful to all of the authors who have contributed generously of their time and energies to make this volume a reality.

To metastasize, neoplastic cells dissociate from the primary tumor, enter a circulatory compartment (typically lymphatics or blood vasculature), survive transport, arrest, exit the circulation and finally proliferate at a discontinuous site in response to local growth factors. Unless cells accomplish *every* step of the metastatic cascade, metastases cannot develop. The process is highly inefficient, i.e., <0.1% of cells entering the vasculature form clinically detectable secondary tumors. At each step of the metastatic cascade, multiple genes and proteins are involved. Because inappropriate movement of cells with subsequent colonization of secondary sites implies that some of those genes are either mutated or aberrantly regulated, it follows that identifying and manipulating metastasis-regulatory genes could lead to decreased efficiency of the metastatic process and better systemic control of neoplasia. Moreover it must be emphasized that each of the genetic defects responsible for developing metastatic potential is superimposed over those already involved in the genesis of a tumor. A paradigm describing the genetics of progression toward metastasis may be modeled after the oncogene and tumor suppressor gene paradigm in the development of carcinomas. Analogous to the role of oncogenes in tumorigenesis, metastasis-promoting genes drive conversion from nonmetastatic to metastatic. Similar to tumor suppressor genes, metastasis suppressors would inhibit the metastatic process. In the case of negative regulators, the distinction between tumor suppressors and metastasis suppressors is critical! Tumor suppressors, by definition also block metastasis since tumor formation is a prerequisite to metastasis. However, using this functional definition, metastasis suppressors only block spread to distant sites. They do not reduce tumor formation.

To date, only a limited number of genes have been shown to functionally regulate the metastatic cascade; but, fortunately, the number of genes identified is growing rapidly. Our criteria for claiming a role in metastasis requires *in vivo* validation. Simply put, *in vitro* surrogates of component steps of the metastatic

cascade are inadequate to measure a complex, multistep, multigenic phenotype like metastasis.

The ability to metastasize can be due to inherent deficiencies within tumor cells themselves (i.e., genetics) or to defective responses to the host environment (i.e., epigenetics). The relative contribution of each has yet to be fully determined. But because of the currently available technologies, the former will be the focus of this volume. Within this volume, several candidate metastasis-regulatory genes are described in detail. The chapters are organized into loose sections. Because the field of metastasis genetics is still in its infancy, the clusters are somewhat arbitrary and artificial. However, they provide one attempt to overview this rapidly expanding area of research.

Yoshida and colleagues review the emerging field of cancer metastasis genetics, highlighting the context under which the genes were discovered and how they fit into a larger picture. This chapter is followed by discussion of genes which promote tumor progression (i.e., metastasis-promoting genes). Identification of metastasis-promoting genes is notoriously difficult because of the inherent nature of the metastatic process. Since only one step needs to be blocked in order to prevent metastasis, introduction of a *bona fide* metastasis-promoting gene into a cell would not necessarily enhance metastasis if that cell were defective for another step. Hence, the model system from which one starts is critical. Alessandro Alessandrini highlights components of the MAP kinase signaling cascade and how they confer tumorigenic and metastatic potential upon NIH3T3 cells. Garth Nicolson and colleagues describe a recently discovered gene mta1, which promotes metastasis. mta1 appears to be involved in regulation of gene expression, perhaps downstream of such signaling pathways.

Peter Brooks summarizes the environmental milieu in which tumor cells reside and the importance of surface adhesion molecules in mediating the metastatic phenotype. Outside-in signaling is critical in the efficiency of metastasis. Coupling the surface molecules to intracellular events (as highlighted above) will be an area of fruitful future research. In a similar vein, Dario Marchetti describes an example for organ-specific melanoma metastasis to brain. Identification of neurotrophins represents a class of exogenous signals which contribute to metastasis. They remain some of the few well-defined molecules which can explain why some cells colonize certain organs while others do not. While tumor cell behavior can be modulated by environment, they sometimes carry the necessary machinery themselves. Onishi and colleagues describe roles for autocrine signals of motility as contributors to metastasis.

The majority of chapters herein describe roles for metastasis-suppressor genes. Since the discovery of Nm23 by Patricia Steeg in 1984, the list of metastasis suppressors has grown significantly to include AP2, KiSS1, BRMS1, and MKK4 among others. Menashe Bar-Eli describes a role for the transcriptional regulator AP2 in melanoma metastasis, including how this gene regulates the expression of other

metastasis-associated genes. Dawn Kirschmann and Mary Hendrix provide evidence that heterochromatin associated protein ($HP1^{HS\alpha}$) might regulate gene expression critical for metastasis. Both of these genes make logical candidates for metastasis regulation since each may be involved in coordinated regulation of expression of multiple critical genes. Likewise, Gary Meadows and colleagues present some intriguing data which shows that diet can modulate metastatic potential. Specific amino acid deprivation can markedly inhibit metastasis. They hypothesize that amino acid response elements may be controlling families of metastasis-regulatory genes.

Carrie Rinker-Schaeffer's laboratory has described a metastasis suppressor effect with a member of the stress-activated MAP kinase family. Like the MAP kinase family and the promotion of metastasis, the downstream effectors of MKK4 are not yet known. As these become more finely defined, crosstalk and feedback mechanisms will likely emerge. These pathways will be important as the field begins to dissect whether there are metastasis suppressors which function universally (i.e., for all tumor types) or whether there are metastasis suppressor genes which act only upon one tumor type (e.g., breast cancer, but not prostate cancer or melanoma).

Thus far, most metastasis suppressor genes have been identified and tested using only a limited number of models. Two examples from the Welch laboratory are described. BRMS1 was isolated from breast carcinoma cells inhibited for metastasis following introduction of human chromosome 11. The mechanism of action is speculated upon by Rajeev Samant, but thus far appears to be involved in transcriptional regulation of other genes. KiSS1 was discovered in human melanoma cells suppressed for metastasis following introduction of chromosome 6. John Harms summarizes the current knowledge of the KiSS1 gene and the steps of the metastatic process inhibited in the cells from which this gene was derived. The chromosome 6 hybrid cells complete every step of the metastatic cascade, except growth at the secondary site. The mechanisms of action for all of the metastasis regulatory genes remain largely enigmatic, but this result provides one small example of the types of progress being made. Ann Chambers and colleagues have utilized the powerful technique of intravital video microscopy to challenge the notion that metastatic cells must extravasate in order to colonize an organ. While it is far from certain whether intravascular or perivascular proliferation of metastases is more prevalent, the implications regarding the genetic control of the metastatic phenotype must take both possibilities into account.

Finally, Jeremy Graff and Steve Zimmer review an understudied area with regard to metastasis regulation B translation. They provide some compelling data highlighting correlations between translational efficiency and metastasis.

Together these chapters provide a glimpse into the complex world of metastasis genetics. Certainly, there remains a great deal of work to be done. Yet, the future looks bright for translating the types of research represented herein to the clinic.

LIST OF CONTRIBUTORS

Allessandrini, Allessandro. Medical Services, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, U.S.A.

Bar-Eli, Menashe. University of Texas, M.D. Anderson Cancer Center, Department of Cancer Biology, Houston, Texas, U.S.A.

Brooks, Peter C. New York University School of Medicine, Kaplan Cancer Center, Departments of Radiation Oncology and Cell Biology, New York, NY 10016, U.S.A.

Chambers, Ann F. London Regional Cancer Centre, London, Ontario N6A 4L6, Canada.

Clare, Susan E. Women's Cancers Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD; Division of Surgical Oncology, Department of Surgery, Northwestern University, Chicago, IL, U.S.A.

Dubauskas, Zita. The University of Chicago, Section of Urology and Genitourinary Oncology Research Program, Chicago, Illinois, U.S.A.

Fu, Ya-Min. Cancer Prevention and Research Center, Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164-6510, U.S.A.

Ge, Xiaokang. Cancer Prevention and Research Center, Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164-6510, U.S.A.

Graff, Jeremy R. Lilly Research Laboratories, Cancer Research Division, Eli Lilly and Company, Indianapolis, IN, U.S.A.

Haga, Arayo. Karmanos Cancer Institute, Wayne State University, Detroit, MI, U.S.A.

Harms, John F. Jake Gittlen Cancer Research Institute, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, U.S.A.

Hartsough, Melanie T. Women's Cancers Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD; Division of Surgical Oncology, Department of Surgery, Northwestern University, Chicago, IL, U.S.A.

Hendrix, Mary J.C. Department of Anatomy & Cell Biology, Holden Cancer Center at the University of Iowa, College of Medicine, Iowa City, Iowa, 52242-1109, U.S.A.

Kirschmann, Dawn A. Department of Anatomy & Cell Biology, Holden Cancer Center at the University of Iowa, College of Medicine, Iowa City, Iowa, 52242-1109, U.S.A.

Mair, Michael. Women's Cancers Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD; Division of Surgical Oncology, Department of Surgery, Northwestern University, Chicago, IL, U.S.A.

Marchetti, Dario. Department of Neurosurgery, The University of Texas-Houston Health Science Center, Houston, TX, U.S.A.

Meadows, Gary G. Cancer Prevention and Research Center, Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164-6510, U.S.A.

Nawa, Akihiro. Department of Obstetrics and Gynecology, Nagoya University School of Medicine, Nagoya 466, Japan.

Nicolson, Garth L. The Institute of Molecular Medicine, Huntington Beach, California 92649, U.S.A.

Nishimori, Katsuhiko. Department of Obstetrics and Gynecology, Nagoya University School of Medicine, Nagoya 466, Japan.

Onishi, Yasuharu. Karmanos Cancer Institute, Wayne State University, Detroit, MI, U.S.A.

Oros, Daniel R. Cancer Prevention and Research Center, Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164-6510, U.S.A.

Ouatas, Taoufik. Women's Cancers Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD; Division of Surgical Oncology, Department of Surgery, Northwestern University, Chicago, IL, U.S.A.

Raz, Avraham. Karmanos Cancer Institute, Wayne State University, Detroit, MI, U.S.A.

Rinker-Schaeffer, Carrie W. The University of Chicago, Section of Urology and Genitourinary Oncology Research Program, Chicago, Illinois, U.S.A.

Samant, Rajeev S. Jake Gittlen Cancer Research Institute, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, U.S.A.

Shevde, Lalita R. Jake Gittlen Cancer Research Institute, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, U.S.A.

Sokoloff, Mitchell H. The University of Chicago, Section of Urology and Genitourinary Oncology Research Program, Chicago, Illinois, U.S.A.

Steeg, Patricia S. Women's Cancers Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD; Division of Surgical Oncology, Department of Surgery, Northwestern University, Chicago, IL, U.S.A.

Taniguchi, Shigeki. Laboratory of Molecular Biology, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan.

Toh, Yasushi. Department of Gastroenterorogic Surgery, National Kyushu Cancer Center, 811-1395 Japan.

Tuck, Alan B. London Regional Cancer Centre, London, Ontario N6A 4LS, Canada.

Welch, Danny R. Jake Gittlen Cancer Research Institute, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, U.S.A.

Yoshida, Barbara A. The University of Chicago, Section of Urology and Genitourinary Oncology Research Program, Chicago, Illinois, U.S.A.

Zhang, Hui. Cancer Prevention and Research Center, Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164-6510, U.S.A.

Zimmer, Stephen G. L.P. Markey Cancer Center, University of Kentucky, Department of Microbiology and Immunology, Lexington, U.S.A.

TABLE OF CONTENTS

Preface vii
Danny R. Welch

List of Contributors xi

Chapter 1 1
Metastasis-Suppressor Genes: A Review and Perspective on an Emerging Field
Barbara A. Yoshida, Zita Dubauskas, Mitchell H. Sokoloff, Danny R. Welch, Carrie W. Rinker-Schaeffer

Chapter 2 35
The Roles of Map Kinases in Controlling Cancer Metastasis
Alessandro Alessandrini

Chapter 3 51
Tumor Metastasis-associated Human Mta1 Gene: Role in Epithelial Cancer Cell Proliferation and Regulation
Garth L. Nicolson, Akihiro Nawa, Yasushi Toh, Shigeki Taniguchi, Katsuhiko Nishimori

Chapter 4 65
Cooperative Integrin Interactions in the Regulation of Tumor Metastasis
Peter C. Brooks

Chapter 5 89
Brain Metastasis Associated Genes
Dario Marchetti

Chapter 6 109
Autocrine Motility Factor and its Receptor as Regulators of Metastasis
Yasuharu Onishi, Arayo Haga, Avraham Raz

Chapter 7 123
nm23 Metastasis Suppressor Gene
Patricia S. Steeg, Taoufik Ouatas, Michael Mair, Susan E. Clare, Melanie T. Hartsough

Chapter 8 145
Gene Regulation in Melanoma Metastasis
Menashe Bar-Eli

Chapter 9 169
Heterochromatin-associated Protein 1, $HP1^{Hs\alpha}$, in Breast Cancer Invasion and Metastasis
Dawn A. Kirschmann, Mary J.C. Hendrix

Chapter 10 191
Inhibition of Invasion and Metastasis During Specific Amino Acid Restriction Associated with Metastasis Suppressor and Other Gene Changes
Gary G. Meadows, Xiaokang Ge, Hui Zhang, Daniel R. Oros, Ya-Min Fu

Chapter 11 209
Role of BRMS1 in Breast Carcinoma Metastasis
Rajeev S. Samant, Lalita R. Shevde, Danny R. Welch

Chapter 12 219
The Role of KISS1 in Melanoma Metastasis Suppression
John F. Harms, Danny R. Welch

Chapter 13 231
Osteopontin: a Ras-regulated Gene That Contributes to Tumor Metastasis
Ann F. Chambers and Alan B. Tuck

Chapter 14 247
The Emerging Role for the mRNA Cap-binding Protein, EIF-4E, in Metastatic Progression
Stephen G. Zimmer and Jeremy R. Graff

Index 267

Chapter 1

METASTASIS-SUPPRESSOR GENES: A REVIEW AND PERSPECTIVE ON AN EMERGING FIELD

Barbara A. Yoshida[1], Zita Dubauskas[1], Mitchell H. Sokoloff[1], Danny R. Welch[2], Carrie W. Rinker-Schaeffer[1]

[1]*The University of Chicago, Section of Urology and Genitourinary Oncology Research Program, Chicago, Illinois* [2]*Pennsylvania State University College of Medicine, Hershey, Pennsylvania.*

Abstract

Metastasis is the most lethal attribute of a cancer. There is a critical need for markers that will distinguish accurately those histologic lesions and disseminated cells with a high probability of causing clinically important metastatic disease from those that will remain indolent. While the development of new diagnostic markers of metastasis was the initial motivation for many studies, the biologic approach used to identify metastasis-suppressor genes has provided surprising insights into the *in vivo* mechanisms regulating the formation of metastases. This chapter reviews the evolving view of the mechanisms that regulate metastasis and the importance of metastasis-suppressor genes in this process. The known metastasis-suppressor proteins or genes and the microcell-mediated chromosomal transfer strategy used to identify many of them are reviewed. New evidence for the role of these metastasis-suppressor activities (genes) in regulating the growth of disseminated cancer cells at the secondary site, the potential for the identification of novel therapeutic targets, and the multidisciplinary approach needed to translate this information into clinical tools for the treatment of metastatic disease are discussed.

1. THE CLINICAL PROBLEM: PREDICTING METASTATIC PROPENSITY

Our ability to detect and successfully treat localized cancers has improved appreciably in recent years. However, metastatic disease presents a continuing therapeutic challenge and is the most common cause of cancer-related death. Thus, there is an emphasis on the diagnosis of cancers at an early stage, when they are localized and most likely to be curable. Although screening for early stage disease is logical, its utility is limited by the inability of conventional diagnostic and histologic parameters to predict accurately the true extent and prognosis of a substantial proportion of clinically localized cancers (1-3). This

D.R. Welch (ed.), Cancer Metastasis – Related Genes, 1–33.

limitation is due, in part, to the inherent limitations and subjectivity of current grading and staging systems (4, 5).

The incidence of disease recurrence in surgical patients treated for prostatic and breast cancer illustrates this problem particularly well. Although we have a wealth of clinical and biologic information on these diseases, a large percentage of apparently resectable and theoretically curable lesions are found to be more advanced at the time of resection than envisaged, resulting in a substantial failure rate after attempted curative surgery (6-8). In studies of prostate cancer patients, even when patient selection excludes men with factors predicting poor prognosis (e.g. poorly differentiated histology, high prostate specific antigen [PSA] levels, clinical suspicion of local invasion) the relapse rate after radical retroperitoneal prostatectomy has approached 20%-30% (9-11). Similarly, one-third of surgical patients with node-negative breast cancer will develop metastases, while the other two-thirds, despite receiving no chemotherapy, do not (12). Even in patients with small tumors and tumor-negative lymph nodes (T1N0), there is a 15 to 25% likelihood of distant metastases (8).

Since the current staging systems for breast and prostate cancers do not accurately identify those patients curable by regional treatment alone, the evaluation of additional parameters associated with the metastatic phenotype will be very important for the differentiation of patients curable by surgery alone from those requiring systemic therapy. For instance, men at high risk for relapse of prostate cancer can be identified (e.g. serum PSA > 10 ng/ml, clinical stage T_1 or T_2 with greater than 50% of tissue at Gleason grade 4 (3, 4) on biopsy or clinical stage T_3 prostate cancer) and would be immediate candidates for adjuvant anti-metastatic therapies if they existed (10, 11, 13-16). Likewise, breast cancer patients with particularly poor prognoses can be identified by the detection of high microvessel counts concurrent with low expression of Nm23 and/or E-cadherin in the primary tumor (12-17). In fact, these parameters are better prognostic biomarkers than the conventional analysis of tumor size and grade. The information obtained from the simultaneous evaluation of biomarkers such as these have the potential to lead to a reduction in the morbidity in those patients not requiring chemotherapy, and possibly identify those patients requiring more aggressive therapies than indicated by current methods.

Overall, it is clear that there is a critical need for markers that will distinguish accurately those histologic lesions and disseminated cells that have a high probability of causing clinically important metastatic disease from those that will remain indolent (5, 15). Concerns have been raised that "metastasis" has often occurred by the time of diagnosis of the primary tumor, the implication being that it is then too late for anti-metastatic therapy to be of use (18). However, the mere spread of cancer cells into the vasculature or to a secondary site does not constitute metastasis. Development of clinically significant metastases requires that a cancer cell complete a series of well-defined steps,

generally referred to as the metastatic cascade (13). If a cell fails to complete any one of these steps, overt metastases will not develop (13-15).

The clinical importance of disseminated cancer cells (detected by sensitive methods such as reverse transcriptase polymerase chain reaction [RT-PCR]) has become an issue of considerable interest (19). Several such studies have reported the detection of tumor-derived cells in the circulation and bone marrow without future development of disease (16, 20). Other reports have demonstrated an increased risk of disease recurrence in patients with bone marrow micrometastases both for prostate cancer (by the detection of mRNA transcripts for prostate specific antigen [21]), and breast cancer (by the detection of cytokeratin-positive cells [22]). Even in these later studies, however, the majority of patients with tumor cell-positive bone marrow samples did not actually develop recurrent disease, although the proportion with recurrence could increase given extended time for patient follow-up. The discrepancy regarding the clinical importance of disseminated cells is likely due to differences in the experimental approaches used to identify cells (i.e. RT-PCR vs. immunohistochemical detection).

Tumor-cell growth at the site of metastasis is an important clinical target since cells must survive and proliferate in order to grow into overt, macroscopic metastases. The first step toward developing effective therapies to inhibit such growth is to identify the genes/proteins that regulate metastatic colonization. To this end, a growing number of laboratories are focusing translational research efforts on the discovery of genes that specifically regulate the metastatic ability of cancer cells. For example, several metastasis-promoting genes – including *WDNM-1*, *WDNM-2*, *MMP11* (stromelysin-3), *MTA1*, and *ERBB2* – have been identified in association with the development of metastatic breast cancer (23-27). One must keep in mind, however, that it takes the coordinated expression of many genes to allow the development of metastases (28, 29). Thus, while it is relatively easy to demonstrate an association for a given gene with metastatic ability, it is difficult to prove that a particular gene is essential. On the other hand, it only takes one gene to block metastasis since inability to complete any step of the metastatic cascade renders a cell nonmetastatic. Metastasis-suppressor genes suppress the formation of spontaneous, macroscopic metastases *without affecting the growth rate of the primary tumor*. It has now been more than ten years since the discovery of the first metastasis-suppressor gene *nm23* (NME1) (30). Since then, both *in vitro* and *in vivo* (eg. animal) studies have documented the important role of the loss of metastasis-suppressor gene function in the acquisition of metastatic ability (15, 30-32).

While the initial motivation for these studies was the development of new diagnostic markers of metastasis, the biologic approach used to identify metastasis-suppressor genes has provided surprising insights into the *in vivo* mechanisms regulating the formation of metastases. We anticipate that

identifying the molecular pathways that regulate metastatic colonization and growth control at the secondary site will provide additional, potentially novel therapeutic targets for the treatment of metastatic disease. The purpose of this chapter is to:

- Present the evolving view of the mechanisms that regulate metastasis
- Describe the functional strategy used to identify metastasis-suppressor genes and discuss important principles learned from these studies
- Document the known metastasis-suppressor genes and report new evidence that supports their role in the regulation of growth control at the secondary site
- Discuss the multidisciplinary approach needed to translate metastasis-suppressor genes into clinical tools

1.1 Regulation of Metastatic Propensity – Evolving Paradigms

Metastasis is defined as the formation of progressively growing secondary tumor foci at sites discontinuous from the primary lesion (15). This process is illustrated by the spontaneous hematogenous metastasis of tumor cells to the lung (Figure 1, Panel A). The formation of a primary tumor requires a cadre of molecular and cellular alterations that enable a cell(s) to circumvent normal growth control mechanisms, as well as, to manipulate its local environment (14). These changes include the development of a blood supply once the focus of transformed cells grows beyond a size that can be nourished by nutrient or metabolite diffusion (33, 34). Tumor progression and the acquisition of metastatic competence requires additional changes in gene expression (e.g. protein degrading enzymes, adhesion molecules) that culminate in a malignant phenotype. Following invasion into adjacent tissues, tumor cells disseminate via blood vasculature or lymphatics and travel individually or as emboli comprised of tumor cells or tumor and host cells. At the secondary site, cells or emboli arrest either because of their physical size or by binding to specific molecules in particular organs or tissues (15, 35). In order for disseminated cells to grow into overt metastases, they must survive and proliferate in the vasculature or in the surrounding tissue after extravasation. The formation of clinically important metastases depends upon the completion of *every step of this cascade*, the last of which is metastatic colonization (Figure 1) (14).

The presence of isolated cells at a secondary site represents a risk to the patient. Cells getting to the secondary site certainly have the potential to colonize, and therefore, it is crucial not to ignore the presence of neoplastic cells anywhere. On the other hand, as we will show, the mere presence of cells does not necessarily mean that metastatic colonization will occur. The challenge is to determine how to discriminate between disseminated cells that will form overt metastases from those that will not.