Critical Factors in the Biology of Human Cancer Metastasis: Twenty-eighth G. H. A. Clowes Memorial Award Lecture

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Abstract

The process of metastasis is not random. Rather, it consists of a series of linked, sequential steps that must be completed by tumor cells if a metastasis is to develop. Although some of the steps in this process contain stochastic elements, as a whole, metastasis favors the survival and growth of a few subpopulations of cells that preexist within the parent neoplasm. Moreover, metastases can have a clonal origin, and different metastases can originate from the proliferation of single cells. The outcome of metastasis depends on the interaction of metastatic cells with different organ environments. Organ-specific metastases have been demonstrated in a variety of experimental tumor systems. Moreover, we have found tumor growth that is specific to a particular site within one organ. Whether the same conclusions can be reached for human cancers remained unanswered until very recently.

Studies from our laboratory and from others have shown that the implantation of human cancer cells derived from surgical specimens into correct anatomical sites of nude mice can provide a suitable model of metastasis of human tumors. Clonal analysis of a human renal carcinoma, colon carcinomas, and melanomas has revealed that these tumors are indeed heterogeneous for metastatic properties, an observation made only after orthotopic implantation. Thus, growth in the environment of specific organs can be selective and the environment per se influences this process.

While it is clear that vascularity and local immunity can facilitate or retard tumor growth, we have concentrated on understanding how damage to an organ and the subsequent repair process can facilitate tumor cell proliferation. Accelerated growth of human colon cancer cells was found in hepatectomized nude mice, whereas accelerated growth of human renal cancer cells was found in nephrectomized nude mice. These data suggest that systemic physiological signals can be recognized by neoplastic cells presumably by mechanisms similar to those shared by their normal cell counterparts. In summary, the critical factors that regulate metastasis are the intrinsic properties of metastatic cells and host factors involved in homeostasis. The recent increase in our understanding of metastasis should provide important leads for developing more effective approaches to the treatment of disseminated cancer.

Introduction

To be a recipient of the G. H. A. Clowes Memorial Award is a distinct honor which I shall always cherish. I consider myself a representative of a larger group of scientists whose endeavors in elucidating the mysteries of cancer metastasis you have recognized. During the last 20 years, I have devoted myself to the understanding of the biology and therapy of cancer metastasis. My interest in this field was stimulated by my teacher, Irving Zeidman, who taught me to ask relevant questions, to search for answers, and to even sometimes ignore dogmas. I am also grateful to many friends, colleagues, and students with whom I have worked. Their contributions will become clear during my presentation. In particular, I thank Margaret Kripke, George Poste, Garth Nicolson, Ian Hart, Avraham Raz, Robert Kerbel, Philip Frost, Michael Hanna, David Tarin, and Frederick Becker for productive collaboration and warm friendship. I also take this opportunity to thank the National Cancer Institute and other funding sources for providing the necessary support without which hypotheses cannot become reality.

I am extremely pleased that Dr. Henry Mihich introduced my presentation. In 1978, when he was the Program Chairman of the Annual Meeting, he introduced me as a speaker on a topic of current interest: "Tumor heterogeneity and the biology of cancer invasion and metastasis." At that time, Garth Nicolson, George Poste, Gloria Heppner, and I, among others, had just begun to understand these processes, and I remember how honored I was to be chosen to describe some of the early findings in this emerging field (1). During the last decade, our collective knowledge has advanced considerably, and in this presentation I shall describe some of the progress accomplished by us and others.

My lecture is divided into three parts. First, I shall discuss the pathogenesis of metastasis and describe some of the mechanisms that regulate this process. Second, I shall describe the development of animal models to study the biology of human cancer metastasis. Finally, I shall describe preliminary data on the role of homeostatic factors in the pathogenesis of metastasis.

Metastasis, the spread of cells from the primary neoplasm to distant sites and their growth there, is the most fearsome aspect of cancer. This fear is well founded. Despite significant improvements in early diagnosis, surgical techniques, general patient care, and local and systemic adjuvant therapies, most deaths from cancer are due to metastases that are resistant to conventional therapies (1–9). In a large number of patients with cancer, metastasis may well have occurred by the time of diagnosis (4, 6, 8, 9). The metastases can be located in different lymph nodes and visceral organs and in various regions of the same organ, thus complicating their treatment. Furthermore, the specific organ environment can modify the response of a metastatic tumor cell to systemic therapy and alter the efficiency of anticancer agents.

The major barrier to the treatment of metastases is the biological heterogeneity of cancer cells in primary and secondary neoplasms. This heterogeneity is exhibited in a wide range of genetic, biochemical, immunologic, and biological characteristics, such as cell surface receptors, enzymes, karyotypes, cell morphologies, growth properties, sensitivities to various therapeutic agents, and ability to invade and produce metastasis (1–5, 10–15). Moreover, it is important to remember the term "cancer" denotes a collection of malignancies, with each cancer of each organ consisting of numerous subsets. This tremendous heterogeneity is probably due to the different etiologies, origins, and selection pressures of different cancers.

Continual empiricism in the treatment of cancer is unlikely to produce significant improvement. Therefore understanding...
the mechanisms responsible for the development of biological heterogeneity in primary cancers and in metastases and the process by which tumor cells invade local tissues and spread to distant organs is a primary goal of cancer research. Only from a better understanding will come the ability to design more effective therapy for different cancers and improvements in the way physicians deal with cancer metastasis. My lecture, therefore, reviews recent data that provide some answers to these difficult questions.

The Pathogenesis of a Metastasis

The process of cancer metastasis consists of a long series of sequential interrelated steps, each of which can be rate limiting since a failure or an insufficiency at any of the steps aborts the process (7). The outcome of the process is dependent on both the intrinsic properties of the tumor cells and the responses of the host; the balance of these interactions can vary among different patients (2, 6, 9). In principle, the steps or events required for the formation of a metastasis are the same in all tumors, and I therefore illustrate the process by using melanoma as the model (Fig. 1).

Major steps in the formation of a metastasis are as follows: (a) after the initial transforming event, either unicellular or multicellular, growth of neoplastic cells must be progressive; (b) extensive vascularization must occur if a tumor mass is to exceed 2 mm in diameter (16). The synthesis and secretion of several angiogenesis factors play a key role in establishing a neovascular network from the surrounding host tissue (17,18); (c) local invasion of the host stroma by some tumor cells could occur by several mechanisms that are not mutually exclusive (19–22). Thin-walled venules, like lymphatic channels, offer very little resistance to penetration by tumor cells and provide the most common pathways for tumor cell entry into the circulation. Although clinical observations have suggested that carcinomas frequently metastasize and grow via the lymphatic system, whereas malignant tumors of mesenchymal origin, e.g., melanoma, more often spread by the hematogenous route, the presence of numerous venolymphatic anastomoses invalidates this belief (23); (d) detachment and embolization of small tumor cell aggregates occurs next; (e) tumor cells that survive the circulation must arrest in the capillary beds of organs; (f) extravasation occurs next, probably by the same mechanisms that influence initial invasion; (g) proliferation within the organ parenchyma completes the metastatic process. To produce detectable lesions, the metastases must develop a vascular network, evade the host immune system (2), and respond to organ-specific factors that influence their growth (24–28). Once they do so, the cells can invade host stroma, penetrate blood vessels, and enter the circulation to produce secondary metastases, the so-called "metastasis of metastases" (7–9).

When I described the metastatic process, I emphasized that only a few cells in a primary tumor can give rise to a metastasis. This is due in part to the elimination of any disseminating tumor cell that fails to complete any step in metastasis. The complexity of the pathogenesis of metastasis explains, in part, why the process is deemed to be inefficient (9, 29). For example, the presence of tumor cells in the circulation does not predict that metastasis will occur, because most tumor cells that enter the bloodstream are rapidly eliminated (30). Using radiolabeled B16 melanoma cells, I observed that by 24 h after entry into the circulation, <1% of the cells are still viable, and <0.1% of tumor cells placed into the circulation survived to produce metastases (30). Observations such as this prompted me to ask whether the development of metastases represents the fortuitous survival and growth of very few neoplastic cells or whether it represents the selective growth of unique subpopulations of malignant cells endowed with special properties. In other words, can all cells growing in a primary neoplasm produce secondary lesions, or do only specific and unique cells possess the appropriate properties that would enable them to survive the potentially destructive journey from the primary tumor to the sites of future metastases? Most recent data indeed show that neoplasms are biologically heterogeneous and that the process of metastasis is selective.

Metastatic Heterogeneity

Cells with different metastatic properties have been isolated from the same parent tumor, thus supporting the hypothesis that not all the cells in a primary tumor can successfully disseminate. Two general approaches have been used to isolate populations of cells that differ from the parent neoplasm in metastatic capacity. In the first, metastatic cells are selected in vivo: tumor cells are implanted s.c., i.m., or into other tissues, or they are injected i.v. into mice, and metastasis is allowed to occur. The metastatic lesions are harvested, and the cells that are recovered can first be expanded in culture or used immediately to repeat the process. The cycle is repeated several times. The behavior of the cycled cells is compared with that of the cells of the parent tumor to determine whether the selection process enhanced metastatic capacity (31), and the increase in metastatic capacity of the recovered cells did not result from the adaptation of tumor cells to preferential growth in a particular organ (32–34). This procedure was originally used to isolate the B16-F10 line from the wild-type B16 melanoma (31). It has also been successfully used to produce tumor cell lines with increased metastatic capacity from many of the experimental tumors tested (35–39).

In the second approach, cells are selected for the enhanced expression of a phenotype believed to be important in one or another step of the metastatic sequence, and then they are tested in the appropriate host to determine whether concomitant metastatic potential has been increased or decreased. This method has been used to examine whether properties as diverse
as resistance to T-lymphocytes (40, 41), adhesive characteristics (42, 43), invasive capacity (36, 44–46), lectin resistance (43, 47, 48), and resistance to natural killer cells (49) are important in metastasis.

One obvious criticism of these studies is that the surviving isolated tumor line may have arisen as a result of adaptive rather than selective processes. The first experimental proof for metastatic heterogeneity in neoplasms was provided by Margaret Kripke and me in 1977 in work with the mouse B16 melanoma (50). Using the modified fluctuation assay of Luria and Delbruck (51), we showed that different tumor cell clones, each derived from individual cells isolated from the parent tumor, vary dramatically in their ability to form pulmonary nodules following i.v. inoculation into syngeneic mice. Control subcloning procedures demonstrated that the observed diversity was not a consequence of the cloning procedure (50).

To exclude the possibility that the metastatic heterogeneity found in the B16 melanoma might have been introduced as a result of the lengthy in vivo and in vitro cultivation, we studied the biological and metastatic heterogeneity in a mouse melanoma induced in C3H mice by chronic exposure to UV B radiation and painting with croton oil (52). One mouse thus treated developed a melanoma designated K-1735. The original K-1735 melanoma was established in culture and immediately cloned (53, 54). In an experiment similar in design to the one described for the B16 melanoma (50), we found that the clones differed greatly from each other and from the parent tumor in their ability to produce lung metastases. In addition to differences in the number of metastases, we also found significant variability in the size and pigmentation of the metastases. Metastases to the lymph nodes, brain, heart, liver, and skin were found in addition to lung metastases; those growing in the brain were uniformly melanotic, whereas those growing in other organs generally were not (27, 53, 54).

To determine whether the absence of metastasis production by some clones of the K-1735 melanoma was a consequence of their immunological rejection by the normal host (55–58), Nabil Hanna and I examined their metastatic behavior in young nude mice (59, 60). In such recipients, the immunological barrier to metastatic cells that also may be highly immunogenic is removed, and antigenic metastatic cells may thus successfully complete the process. Cells of two clones that did not produce metastases in normal syngeneic mice produced tumor foci in the young nude mice. However, most of the nonmetastatic clones were nonmetastatic in both normal syngeneic and nude recipients. Therefore, the failure of the clones to metastasize in syngeneic mice was probably not caused by their immunological rejection by the host but by other deficiencies that prevented completion of one or another step in the complex metastatic process (Fig. 2).

The finding that preexisting tumor cell subpopulations proliferating in the same tumor exhibit heterogeneous metastatic potential has since been confirmed in many laboratories, with a wide range of experimental animal tumors of different histories and histological origins (1–4, 7, 10, 11, 13–15). In addition, studies using young nude mice as models for metastasis of human neoplasms have shown that several human tumor lines and freshly isolated tumors, such as colon carcinoma and renal carcinoma, also contain subpopulations of cells with widely differing metastatic properties (60).

James Talmadge and I also addressed the question of whether the cells that survive to form metastases possess a greater metastatic capacity than most cells in an unselected neoplasm (31, 34, 37). Most lines derived from metastatic deposits produced significantly more metastases than cells of the parent line. Studies with heterogeneous, unselected neoplasms have therefore led us to conclude that metastasis is a selective process regulated by a number of different mechanisms.

Role of the Organ Environment in the Pathogenesis of Metastasis

Clinical observations of cancer patients and studies with experimental rodent tumors have revealed that certain tumors produce metastasis to specific organs independent of vascular anatomy, rate of blood flow, and number of tumor cells delivered to each organ. The distribution and fate of hematogenously disseminated, radiolabeled melanoma cells in experimental rodent systems amply demonstrate that tumor cells reach the microvasculature of many organs (30, 61–63). Extravasation into the organ parenchyma and proliferation of tumor cells occur in only some organs. Therefore, the mere presence of viable tumor cells in a particular organ does not always predict that the cells will proliferate to produce metastases (24–26, 64).

The search for the mechanisms that regulate the pattern of metastasis began a century ago. In 1889, Paget (65) questioned whether the distribution of metastases was due to chance. He therefore analyzed 735 autopsy records of women with breast cancer. The nonrandom pattern of visceral metastases suggested to Paget that the process was not due to chance but, rather, that certain tumor cells (the “seed”) had a specific affinity for the milieu of certain organs (the “soil”). Metastases resulted only when the seed and soil were matched (65).

Experimental data supporting the “seed and soil” hypothesis of Paget were derived from studies on the preferential invasion and growth of B16 melanoma metastases in specific organs (66, 67). Ian Hart and I injected B16 melanoma cells into the circulation of syngeneic C57BL/6 mice. Tumor growths developed in the lungs and in fragments of pulmonary or ovarian tissue implanted i.m. In contrast, metastatic lesions did not develop in renal tissue implanted as a control or at the site of surgical trauma (67). This study confirmed that sites of metastasis are determined not solely by the characteristics of the neoplastic cells but also by the microenvironment of the host tissue. In vitro experiments demonstrating organ-selective adhesion, invasion, and growth also support Paget’s hypothesis (65).

With the B16 melanoma system, cells that are selective for organ adhesion, invasion, and growth have been isolated (24, 26, 31–39, 68). Moreover, experiments with organ tissue-derived soluble growth factors indicate that soil factors can have profound effects on certain tumor cell subpopulations (24). There is no question that the circulatory anatomy influences
the dissemination of many malignant cells (6, 8, 9); however, it cannot, as Ewing proposed (69), fully explain the patterns of distribution of numerous tumors. Ethical considerations rule out the experimental analysis of cancer metastasis in patients as studied in laboratory animals, by which either Paget or Ewing might be proved correct. The introduction of peritoneovenous shunts for palliation of malignant ascites has, however, provided an opportunity to study some of the factors affecting metastatic spread in humans. David Tarin and colleagues have described the outcome in patients with malignant ascites draining into the venous circulation, with the resulting entry of viable tumor cells into the jugular veins (70, 71). Good palliation with minimal complications was reported for 29 patients with different neoplasms. The autopsy findings in 15 patients substantiated the clinical observations that the shunts do not significantly increase the risk of metastasis. In fact, despite continuous entry of millions of tumor cells into the circulation, metastases in the lung (the first capillary bed encountered) were rare (70, 71). These results provide compelling verification of the venerable “seed and soil” hypothesis (65).

An interesting demonstration for organ-specific metastasis comes from recent studies. Gabriele Schackert and I described the development of a mouse model with which to study cerebral metastasis after injection of syngeneic tumor cells into the internal carotid artery (72, 73). A direct, i.e.2 injection of tumor cells was used to determine tumorigenicity. The injection of cells into the internal carotid artery of mice simulates the hematogenous spread of tumor emboli to the brain. Thus, this technique can examine the last steps of the metastatic process: release of tumor cells into the circulation; arrest of tumor cells in capillaries; penetration and extravasation of the tumor cells into the brain through the blood-brain barrier; and continuous growth of the cells in the tissue.

We found a remarkable difference between two murine melanomas in patterns of brain metastasis; the K-1735 melanoma produced lesions only in the brain parenchyma, whereas the B16 melanoma produced only meningeal growths (73). These results demonstrate specificity for metastatic growth in different regions within a single organ. The results from site distribution analysis of radiolabeled murine melanoma cells injected into the internal carotid artery ruled out that the patterns of initial cell arrest in the microvasculature of the brain predicted the eventual sites of growth. Thus, an alternative explanation for the different sites of tumor growth involves interactions between the metastatic cells and the organ environment, possibly in terms of specific binding to endothelial cells and responses to local growth factors. In other words, organ-specific metastases are produced by tumor cells that are receptive to their new environment.

Models for Human Cancer Metastasis

An appropriate model for studies of human cancer metastasis must meet two rigid demands: it must use metastatic cells (seed), and it must grow in the relevant organ environment (soil). In 1983, when I joined the faculty of the University of Texas M. D. Anderson Cancer Center, I began a collaborative study with Drs. Kim Jessup, Raffaella Giavazzi, and later Kiyoshi Morikawa to design a bioassay of the malignant potential of single-cell suspensions obtained by enzymatic dissociation of the different sites of tumor growth involves interactions between the metastatic cells and the relevant organ environment. In this way, organ-specific metastases can be used to ascertain the metastatic potential of allogeneic tumors and that metastatic variants can be selected in nude mice from heterogeneous mouse tumors (75). We therefore decided to use similar techniques to investigate the metastatic potential of HCC. We reasoned that if a HCC contained metastatic cells, these cells would produce metastasis in nude mice, whereas HCCs that do not contain metastatic cells will not. As you shall soon see, this was a gross oversimplification.

Correlation of Experimental Metastatic Behavior with Clinical Staging

In initial studies, four tumor lines were derived from primary HCCs, three from hepatic metastases and one from a mesenteric lymph node metastasis. Tumor cells of each line were injected into multiple sites in nude mice: the spleen; the subcutis; muscle; and the venous system (76, 77). All the inoculi consisted of single-cell suspensions obtained by enzymatic dissociation of solid tumors. In the course of these experiments, we examined by autopsy approximately 600 mice that had growing HCC. Injection s.c., although successful in initiating local tumor growth in only one case yielded visceral metastasis to the lung. In only 10 did histological examination reveal tumor growth in lymph nodes draining the injection site (76, 77).

Metastases of colorectal cancer may occur late in the disease, often after surgical resection of the primary tumor. In some reports of experimental tumor systems in rodents, multiple metastases developed subsequent to surgical removal of a local tumor. Similarly, the incidence of lung metastases was shown to increase with the prolonged survival of nude mice that had locally recurrent HCC at the site of injection—resection (78). In our experiments with nude mice, we injected HCC cells into a hind thigh and amputated the leg when the tumors reached 1.5–2 cm. Although most of the mice developed recurrent tumor at the incision site, metastases were found in lungs of only two mice, even though all mice survived for 6 months after the initial tumor cell injection. Neither cells from primary HCCs nor cells from metastases produced metastasis in nude mice subsequent to s.c. or i.m. implantation. When the HCC cells were administered i.v., no correlation was found between the experimental lung metastases and the clinical stage of the original neoplasms.

We next made a critical decision. Hepatic metastases account for many of the deaths from colorectal carcinoma. To develop a reproducible model of hepatic metastasis, tumor cells have been implanted into the spleens of nude mice. From this site of injection, tumor cells gain access to the blood stream and then reach the liver to proliferate into secondary tumor colonies. In our laboratory, James Kozlowski and coworkers (79) investigated the metastatic behavior of 11 human cell lines of different histological origin and the production of lung and liver metastases in the nude mouse. The extent of metastasis depended on the nature of the tumor cells, with the most dramatic expression of malignancy found for two variants of the HT-29 HCC cell line subsequent to i.s. injection (79). Merely implanting human tumor cells into the spleens of nude mice does not guarantee that metastasis to the liver will occur. A more recent study from

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2 The abbreviations used are: i.e., intracranial; i.s., intrasplenic; HCC, human colon carcinoma; CEA, carcinoembryonic antigen; HRCC, human renal cell carcinoma.
our laboratory demonstrated that variant lines established from a surgical specimen of a human renal cell carcinoma produced extensive metastasis if the cells were implanted into the kidney of nude mice. In contrast, i.s. implantation of these cells produced only splenic tumors and not metastasis (80).

The i.s. injection of HCC cells followed by the formation of tumor lesions in the liver allowed us to distinguish HCC with different malignant potentials. Thirty days after the injection of tumor cells derived from liver metastases of HCC (Dukes' stage D), the mice became moribund and were then killed. At autopsy, their livers were completely replaced by tumor. Mice given injections of cells from primary colorectal carcinomas developed few visible tumor foci in the liver by 90 days after i.s. injection. Cells of one primary tumor produced visible liver tumor in all the injected mice, but this required 90 days (76, 77). The cells recovered from the liver lesions were of human origin (karyotype and isoenzyme analyses).

The previous studies demonstrated that the i.s. injection of HCC cells can lead to the production of discrete tumor nodules in the liver. To further delineate the malignancy of tumors of different origin, Kiyoshi Morikawa compared the behavior of HCC cells enzymatically dissociated from surgical specimens of a primary HCC classified as Dukes' stage, B2, a primary HCC classified as Dukes' stage D, and one liver metastasis. The cells were implanted into the subcutis or spleen of different nude mice or were established in culture. Tumors developed in both sites of implantation, but hepatic tumor nodules were found only in mice given injections of HCC cells into the spleen (81, 82). Once again, cells from surgical specimen of Dukes' stage D tumors produced a significantly higher number of HCC colonies in the liver of nude mice than cells from the Dukes' stage B tumor.

As I mentioned earlier, we began these studies nearly 5 years ago, a sufficient time to be able to determine whether the production of experimental hepatic metastases in athymic nude mice by HCC correlated with the clinical outcome in patients. HCC cells from 82 patients were injected into groups of nude mice, either in the flank to assess tumorigenicity or into the spleen to produce experimental metastasis in the liver. Growth in mice was recorded and compared with clinicopathological factors and clinical outcome. Growth of HCC in either the flanks or the livers of nude mice was associated with the time to recurrence of disease (postsurgery) in a Wilcoxon analysis. Analysis of the outcome data in a Cox proportional hazards model suggested that there was an interaction between tumorigenicity and metastatic potential of HCC in nude mice and serum carcinoembryonic antigen (CEA) concentration in the patient and the stage of disease. A univariate analysis indicated that both tumorigenicity and metastatic potential of HCC in nude mice were significantly associated with the serum CEA concentration of the patient but not with the other variables such as stage of disease, mucin production, local tissue invasion, or state of differentiation. A subset of 57 patients was operated upon for cure and followed prospectively for up to 61 months. Tumorigenicity in nude mice and experimental metastatic potential were associated with disease recurrence in 23 of these patients. Seventy-eight % of the subset of patients who were operated upon for cure developed liver metastasis. Collectively, the ability of HCC cells isolated from surgical specimens to grow in athymic nude mice correlated with the development of advanced disease in patients (83, 84).

These results, however, were somewhat disappointing because we did not succeed in assessing metastatic potential in every tumor and, therefore, failed in the mission of developing a bioassay to improve prognosis and recommend a course of therapy. We did, however, learn a valuable lesson, the principle of orthotopic transplantation. The orthotopic transplantation of colon tumor has been described for a chemically induced murine adenocarcinoma (85–87). Colon tumor cells were injected into different sites along the small and large intestines of syngeneic mice. The highest rate of tumor take occurred in the cecum, and about 50% of mice with local tumors developed liver metastases. The same tumor implanted s.c. grew locally and produced pulmonary but not hepatic metastases (78, 88).

Kiyoshi Morikawa and I next began experiments with orthotopic implantations to select and isolate cells with increased liver-metasatizing potential from heterogeneous primary HCCs. Cells derived from a surgical specimen of a primary HCC classified as Dukes' stage B2 were directly established in culture or were injected into the subcutis, cecum, or spleen of nude mice. Progressively growing tumors were excised, dissociated, and established in culture. Subsequent to implantation into the cecum or spleen of nude mice, cells from all 4 lines produced only a few liver tumor foci. HCC cells from the few liver metastases were expanded in culture and then injected into the spleen of nude mice to provide a source for further cycles of selection. With each successive in vivo selection cycle, the metastatic ability of the isolated-propagated cells increased. Four cycles of selection yielded cell lines with a very high metastatic efficiency in nude mice (81). In parallel studies using another surgical specimen of a primary HCC classified as Dukes' stage D, we isolated cell lines that were highly metastatic in nude mice. Successive selection cycles for growth in the liver only slightly increased metastatic properties (81).

The metastatic potential of the HCC cells in nude mice was determined by two different assays. The first involved the implantation of cells into the spleen and the production of liver tumor foci (experimental metastasis). The second assay measured the ability of HCC cells to produce lymph node and liver metastases subsequent to implantation into the wall of the cecum (spontaneous metastasis). In general, there was agreement on the results of the two assays; a cell line highly metastatic to the liver after i.e. injection was also highly metastatic to the mesenteric lymph nodes after intracecal injection. Neither assay revealed HCC metastases to the lung (81, 82).

The nude mouse model had been used to select metastatic cells from heterogeneous nonselected murine neoplasms (75). Our present results confirm these findings and show that the nude mouse can be used to isolate and expand metastatic subpopulations of cells from HCCs. The classification of a HCC as Dukes' B tumor denotes that the lesion is confined to the liver and, therefore, failed in the mission of developing a bioassay to improve prognosis and recommend a course of therapy. We did, however, learn a valuable lesson, the principle of orthotopic transplantation. The orthotopic transplantation of colon tumor has been described for a chemically induced murine adenocarcinoma (85–87). Colon tumor cells were injected into different sites along the small and large intestines of syngeneic mice. The highest rate of tumor take occurred in the cecum, and about 50% of mice with local tumors developed liver metastases. The same tumor implanted s.c. grew locally and produced pulmonary but not hepatic metastases (78, 88).

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The裸鼠模型已被用于筛选和扩增具有肝转移潜能的HCC细胞（75）。我们的结果证实了这些发现，并显示裸鼠可以被用于隔离和扩增来自HCCs的转移亚群。HCC的分类为Dukes' B肿瘤表示该病灶仅限于肝脏。
HRCC is not a very common cancer. The prognosis of a patient with this cancer is poor because no effective therapy has been established for advanced stages of this disease. Although several investigators have reported the successful transplantation of HRCC cells into the subcutis of nude mice (89-91), the usefulness of this model has been limited. Like other tumor cells, transplanted HRCC cells rarely metastasize in nude mice, regardless of their degree of malignancy in the patient (60). The finding that the growth rate and incidence of cancer metastasis in nude mice can be increased by manipulation of the route of tumor cell implantation and organ implantation sites (76, 78) prompted Seiji Naito to investigate whether the orthotopic implantation of HRCC into the kidney of nude mice would allow the expression of malignant potential.

The purpose of the initial study was to determine whether the methods for isolating cells from a surgical specimen of a HRCC influence the biological behavior of the cancer cells. HRCC obtained from a surgical specimen was dissociated by enzymatic treatment (80, 92), and cells were plated into culture dishes or injected s.c. and into the kidney of nude mice. A resultant kidney tumor also produced liver metastases and ascites. All tumors growing in different organs of nude mice were then established in culture. The human origin of all five lines was ascertained by karyotypic and isoenzyme analysis. Because the five cell lines of HRCCs were isolated by five different methods (culture, s.c. tumor, renal tumor, liver metastasis from the renal tumor, and ascitic cells from the renal tumor), we asked whether the cell lines exhibited biological heterogeneity, including differences in metastatic potential, and whether different implantation sites in nude mice would influence such behavior.

Cells from all lines were injected s.c., i.p., i.v., i.s., and beneath the renal capsule of nude mice. All the lines were tumorigenic after s.c. or renal subcapsule injection, although the rate of tumor growth varied among the five lines. The metastatic behavior of the cells differed significantly among the 5 lines. Some cells exhibited invasive and metastatic properties regardless of organ site for their implantation (s.c., i.v., i.p., or kidney). In contrast, cells of the other lines were poorly metastatic regardless of the site of implantation. Second, the highest incidence of metastasis by the HRCC lines was produced by tumors growing in the kidney. The injection of cells into the peritoneum, spleen, or subcutis was associated with tumorigenicity but not metastasis (80).

The work with HRCC is important for two reasons. First, the site of implantation of human tumor cells can promote the growth of different subpopulations of cells from a heterogeneous tumor. Cell lines established from subcutaneous tumors or kidney tumors differed in their biological properties, and preliminary cytogenetic analysis by Sen Pathak suggests that each cell line has a distinct karyotype. This finding raises a question as to how human tumor cell lines should be isolated for the study of biology and therapy. Second, the implantation of cells from HRCC cell lines into the kidney of nude mice was associated with invasive and metastatic behavior. In contrast, the s.c. implantation of the same cells was associated with the formation of a dense fibrous capsule surrounding the local tumor. This finding indicates that the appropriate nude mouse model for studying the biology and therapy of human cancers must be based on orthotopic implantation of tumor cells.

Orthotopic versus Ectopic Implantations for Studies of Spontaneous Metastasis

The implantation of human tumor cells into the subcutis of nude mice has been reported by many investigators, but the growing tumors fail to produce metastasis (20). Therefore, even if human tumors growing s.c. in nude mice maintain their original morphological and biochemical characteristics, it is questionable whether the s.c. environment of the nude mouse is the most appropriate site for the growth of human tumors (except skin cancers and melanomas). The ideal in vivo model for studying this disease should allow the interaction of the tumor cells with their relevant organ environment.

The data I just discussed raised the question of which organ site of nude mice should be used for the transplantation of heterogeneous human tumors. Although the routine transplantation of human tumors into the s.c. space of nude mice is relatively easy, it may not yield cell lines that resemble the original human tumor. If a human tumor is biologically heterogeneous, some of its cells may possess a growth advantage, depending on whether it is transplanted to the skin, the ceicum, the liver, or the kidney of nude mice.

Orthotopic implantation in nude mice of human tumor cells recovered from surgical specimens is mandatory for accurate assessment of metastatic potential. This is the case not only with human colon (76, 77, 81, 82, 84, 87, 89, 93, 94) and renal cell carcinomas (80, 92), but also melanomas (into the skin) (95-98), mammary carcinomas (into the mammary fat pad) (99-101), bladder carcinomas (into the bladder wall) (102, 103), prostatic carcinoma (into the peritoneum) (79), pancreatic carcinoma (into the pancreas) (104-108), and lung cancer (into the bronchi) (109). All result in rapid growth of local tumors and, in many instances, in distant metastasis.

In sharp contrast, the implantation of these very same human cancer cells at ectopic sites (usually s.c. or i.m.) results in slow growth of local tumors and only rarely in metastasis. These findings are by no means restricted to human neoplasms in nude mice. Similar data show that the implantation site of mouse neoplasms, such as fibrosarcoma (110), melanomas (111), mammary carcinomas (112), and the Lewis lung carcinoma (113) greatly influences the biological behavior of the neoplasms.

Homeostasis and Cancer Metastasis

For the final (and brief) portion of my presentation, I wish to speculate about a mechanism that may influence the growth and behavior of tumor cells at orthotopic sites. During my lecture, I have emphasized that the outcome of metastasis is determined by both the intrinsic properties of tumor cells and by host factors. The latter are likely to be important in maintenance of self, i.e., homeostasis. Factors that control the processes of organ repair and/or regeneration are known to be organ specific. For example, subsequent to a partial hepatectomy (60%), the liver undergoes rapid cell division termed "regeneration." In a hepatectomized mouse, however, no similar cell division can be found in the kidneys. In contrast, the mouse kidney compensates for unilateral nephrectomy by hypertrophy and hyperplasia, whereas the liver does not regenerate after nephrectomy.

Janet Price, Jerald Killion, Hans Schackert, and I carried out transplantation experiments on human colon carcinomas and human renal cell carcinomas in nude mice that have subse-
frquentely been subjected to either nephrectomy, heptectomy (60%), or abdominal surgery (as a trauma control). The results were intriguing. Human colon carcinoma cells implanted s.c. demonstrated accelerated growth in partially heptectomy-mice but not in heptectomized mice. Human renal cell carcino-
moma cells established as micrometastases in the lung of nude mice underwent a significant growth acceleration subsequent to unilateral heptectomy but not heptectomy. In other words, liver regeneration in nude mice stimulated growth of HCC cells, whereas hypertrophy-hyperplasia of the kidney stimulated the growth of human renal cancer cells. In both studies, the human cancer cells were recent isolates from surgical specimens of human cancers.

These results indicate that metastatic cells can respond to physiological signals produced when homeostasis is disturbed, i.e., the processes of repair and regeneration. Damage to the organs is followed first by inflammation. Subsequent repair then stimulates the growth of normal cells. Tumor cells that either originate from or have an affinity for growth in this particular organ can also respond to these signals.

Conclusions

In my presentation, I have stressed the following four points: (a) the process of metastasis is sequential and selective, with every step of the process containing stochastic elements, and the growth of metastases represents the end point of many lethal events that only few tumor cells can survive; (b) primary neoplasms have heterogeneous metastatic properties, and the outcome of metastasis is determined by the interaction of metastatic cells with various host factors, which include immunity [I was warned not to mention the word macrophage, and I promised not to utter the word macrophage even once (114)]; (c) the orthotopic implantation of human tumor cells into nude mice can provide a model to study the biology and therapy of human cancer metastasis. Perhaps the processes of inflammation and repair subsequent to a trauma stimulate the growth of metastatic cells that either originate from or are capable of growing in the particular organ; (d) the outcome of metastasis is influenced by both the intrinsic properties of the tumor cell (seed) and host factors (soil) (Table 1). Thus, the successful metastatic cell, that 10 years ago I called the “de-cathlon champion” (1), must also be viewed as a cell receptive to its new environment. Indeed, understanding the interaction of metastatic cells with their environment may open a new avenue for therapeutic intervention by inhibiting growth of metastases.

So now once again I ask the question: Is the process of cancer metastasis random or is it selective? The data I presented in this paper conclude that metastasis is a highly selective process that is regulated by a number of different mechanisms. This belief is quite contrary to the once widely accepted notion that metastasis represents the ultimate expression of cellular anarchy. In fact, the view that cancer metastasis is a selective process is an optimistic one. Belief that certain rules govern metastasis implies that elucidation and understanding of these rules will lead to better therapeutic intervention. This is an exciting time because we can now approach research on cancer metastasis on the systemic, cellular, and molecular levels. I am exceedingly fortunate to have been able to contribute to these worthwhile endeavors and eagerly look forward to continued increase in our knowledge.

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