

# Principles and Practice of Genitourinary Oncology

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## CHAPTER 2

# Biology of Metastasis: Studies in Renal Cancer

Colin P. N. Dinney and Isaiah J. Fidler

For many patients with cancer, metastasis has occurred by the time of diagnosis. The major barrier to the effective treatment of their metastases is the biologic heterogeneity of neoplastic cells. This heterogeneity is exhibited in numerous genetic, biochemical, immunologic, and biologic characteristics, such as cell-surface receptors, enzymes, karyotype, cell morphology, growth properties, sensitivities to various therapeutic agents, and ability to invade and produce metastasis.<sup>1-7</sup>

Understanding the mechanisms responsible for the development of the biologic heterogeneity of cancer and the process by which tumor cells invade local tissues and spread to distant organs is a primary goal of cancer research. This review describes the way in which the development of relevant preclinical models for human renal cell carcinoma has facilitated the study of the biology of metastasis.

### PATHOGENESIS OF A METASTASIS

The process of cancer metastasis consists of a series of sequential steps, each of which can be rate limiting.<sup>6</sup> After cellular transformation, either unicellular or multicellular, growth of neoplastic cells is progressive, but extensive vascularization must occur if a tumor mass is to exceed 2 mm in diameter.<sup>8</sup> Local invasion of the host stroma by some tumor cells, the next step, can occur by several mechanisms that are not mutually exclusive.<sup>9</sup> For example, thin-walled venules and lymphatic channels, which offer little resistance to penetration by tumor cells, provide the most common pathways for tumor cell entry into the circulation. Detachment of small tumor cell aggregates and embolization occur next. The tumor cells that survive the circulation must lodge in the capillary beds of organs. Extravasation follows, probably by the same mechanisms that influence initial invasion. Proliferation within the organ parenchyma completes the metastatic process (Fig. 2-1). To produce detectable lesions, the metastases must develop a vascular network, evade the host immune system,<sup>2</sup> and respond to organ-specific factors that influence their growth.<sup>10,11</sup> Once they do so, metastatic cells can invade host stroma, penetrate blood vessels, and enter the circulation to produce further metastases, the so-called "metastasis of metastases."<sup>14,5</sup>

Only a few cells in a primary tumor can give rise to clinical

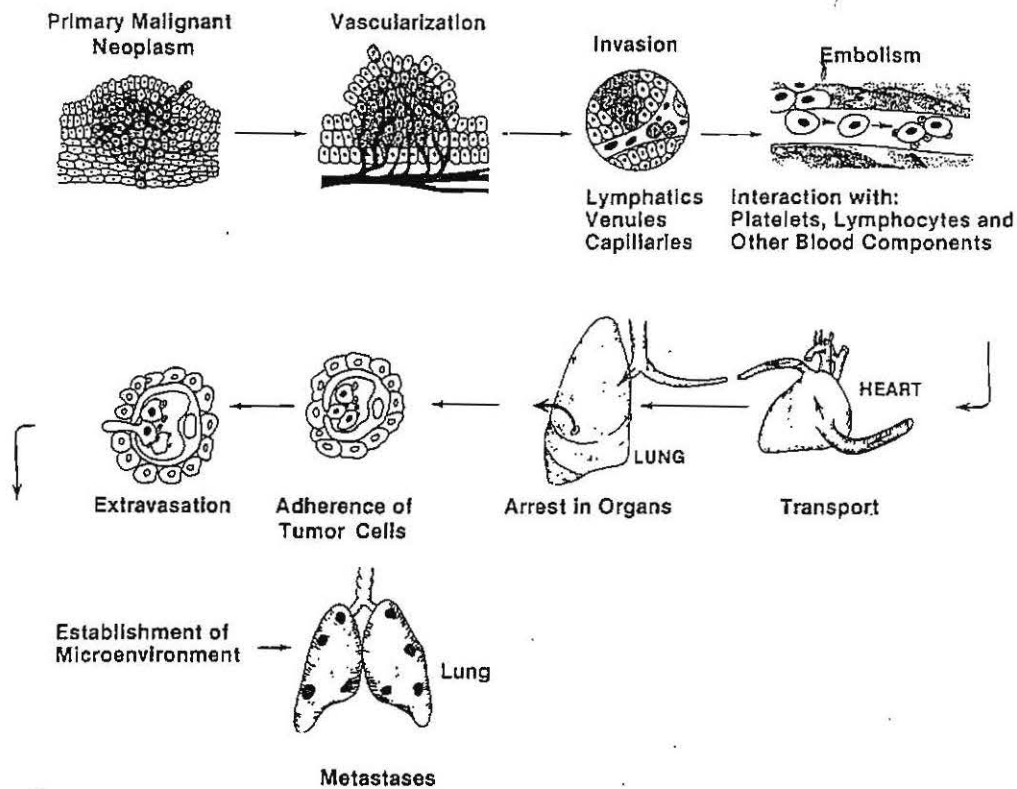
metastasis, partly because of the elimination of disseminating tumor cells that fail to complete any step in metastasis.<sup>12</sup> Using radiolabeled B16 melanoma cells, researchers observed that, by 24 hours after entry of the cells into the circulation, <1% of the cells were still viable, and <0.01% of tumor cells placed into the circulation survived to produce metastases.<sup>12</sup> These observations brought into question whether the development of metastases represents the chance survival and growth of neoplastic cells or the selective growth of unique subpopulations of malignant cells endowed with special properties. In other words, can any cell growing in a primary neoplasm produce metastases, or do only specific and unique cells possess the appropriate properties that enable them to metastasize? Most recent data conclude that neoplasms are biologically heterogeneous and the process of metastasis is indeed selective.

### ROLE OF THE ORGAN ENVIRONMENT

Clinical observations of cancer patients and preclinical studies with experimental rodent tumors have demonstrated that certain tumors produce metastasis to specific organs independent of vascular anatomy, rate of blood flow, or the number of tumor cells delivered to each organ. For instance, 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.<sup>12-15</sup> Extravasation into the organ parenchyma and proliferation of tumor cells occur only in certain organs, however. Therefore, the mere presence of viable tumor cells in a particular organ does not always predict that the cells will proliferate to produce metastases.<sup>10,13-16</sup>

The search for the mechanisms that regulate the pattern of metastasis began more than a century ago when, in 1889,<sup>17</sup> Paget questioned whether the distribution of metastases was due to chance. Paget therefore analyzed 735 autopsy records of women with breast cancer, and the nonrandom pattern of visceral metastases suggested to him that the process was not due to chance, but rather, certain tumor cells (the "seed") had a specific affinity for the milieu of certain organs (the "soil"). Metastases resulted only when the appropriate seed and soil were matched.<sup>17</sup>





**FIG. 2-1.** The pathogenesis of cancer metastasis. To produce clinically relevant metastases, tumor cells in a primary neoplasm must complete a series of sequential, selective, and rate-limiting steps.

Experimental data supporting the “seed and soil” hypothesis came from studies on the preferential invasion and growth of B16 melanoma metastases in specific organs.<sup>18</sup> When the B16 melanoma cells were injected into the circulation of syngeneic mice, tumors developed in the lungs and in fragments of lung or ovarian tissue implanted intramuscularly. In contrast, metastatic lesions did not develop in renal tissue implanted as a control organ or at the site of surgical trauma.<sup>18</sup> This study confirmed that the production of metastasis was determined not only 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.<sup>17</sup> Using the B16 melanoma system, cells with increased capacity for organ adhesion, invasion, and growth have been isolated.<sup>10,19–24</sup> Moreover, experiments with organ tissue-derived soluble growth factors indicate that soil factors can have profound effects on certain tumor cell subpopulations.<sup>10</sup>

Although clinical observations have suggested that carcinomas frequently metastasize through the lymphatic system, and malignant tumors of mesenchymal origin more often spread by the hematogenous route, the presence of numerous venolymphatic anastomoses invalidates this belief.<sup>25</sup> The circulatory anatomy influences the dissemination of many malignant cells; however, it cannot, as Ewing proposed,<sup>26</sup> fully explain the patterns of distribution of numerous tumors. Ethical considerations rule out the experimental analysis of cancer metastasis, as studied in laboratory animals, in patients. The introduction of peritoneovenous shunts for palliation of malignant ascites has, however, provided an opportunity to study some factors affecting

metastatic spread in humans.<sup>27,28</sup> Good palliation with minimal complications was reported for 29 patients with various neoplasms. The autopsy findings in 15 patients substantiated the clinical observations that the shunts do not significantly increase the risk of visceral organ metastasis. In fact, despite continuous entry of hundreds of millions of tumor cells into the circulation, metastases in the lung (the first capillary bed encountered) were unusual.<sup>27,28</sup> These results provide compelling verification of the seed and soil hypothesis.

### METASTATIC HETEROGENEITY

Populations of cells that differ in metastatic potential have been isolated from the parent neoplasm, a finding that supports the hypothesis that not all the cells in a primary tumor can successfully disseminate. Two general approaches have been used. In the first, metastatic cells are selected *in vivo*: tumor cells are implanted into mice, and metastatic lesions are harvested. The cells recovered can be expanded in culture or used immediately to repeat the process. The cycle is repeated several times, and the behavior of the cycled cells is compared with that of the cells of the parent tumor. This procedure was originally used to isolate the B16-F10 line from the wild-type B16 melanoma,<sup>20</sup> and it has since also been successful in producing tumor cell lines with increased metastatic capacity from many other experimental tumors.<sup>6</sup> 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.

The first experimental proof of metastatic heterogeneity in neoplasms was provided by Fidler and Kripke in 1977, in studies with mouse B16 melanoma.<sup>27</sup> Using the modified fluctuation assay of Luria and Delbruck,<sup>28</sup> Fidler and Kripke showed that distinct tumor cell clones, each derived from individual cells isolated from the parent tumor, varied dramatically in their potential to form pulmonary nodules after intravenous inoculation into syngeneic mice. Control subcloning procedures demonstrated that the observed diversity was not a consequence of the cloning procedure.<sup>27</sup>

To exclude the possibility that the metastatic heterogeneity found in the B16 melanoma cells may have been introduced as a result of lengthy cultivation, Kripke studied the biologic and metastatic heterogeneity of a mouse melanoma induced in C<sub>3</sub>H mice by long-term exposure to ultraviolet B radiation and painting with croton oil.<sup>29</sup> One mouse thus treated developed a melanoma designated K-1735. The original K-1735 melanoma was established in culture and immediately cloned.<sup>30,31</sup> The clones differed from each other and from the parent tumor in their ability to produce lung metastases. Moreover, the metastases demonstrated significant variability in their size and pigmentation. 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.<sup>30,31</sup>

## MODELS FOR HUMAN RENAL CANCER METASTASIS

The concept that neoplasms are heterogeneous and contain subpopulations of cells with different patterns of biologic behavior, including metastatic ability, is no longer controversial. Studies in most rodent tumor systems have also demonstrated that metastasis is a selective process,<sup>20,32,33</sup> metastases can have a clonal origin,<sup>34-38</sup> metastases can develop from the expansion of a single cell,<sup>38</sup> and the host organ microenvironment can profoundly influence the growth of metastatic tumor cells.<sup>6</sup> Although most data on the metastatic heterogeneity of neoplasms and on host-tumor cell interactions during the metastatic process have been derived from studies in nonhuman systems, evidence is now accumulating about the biology of metastasis by cells isolated from surgical specimens of human cancers. In large measure, this accumulation of evidence has been due to recent improvements in the use of *in vivo* models for the isolation of metastatic subpopulations of human cancer cells and for testing of their metastatic potential. With the discovery of the athymic T cell-deficient nude mouse, its use as a recipient for human xenografts, and its adaptation for the study of human cancer growth and metastasis,<sup>39</sup> many aspects of the biology of human cancer can now be studied. Reports on the metastatic ability of human tumors subsequent to implantation into nude mice have recently increased,<sup>39,40</sup> and several reports have concluded that the metastatic capacity of human tumor cells in nude mice is influenced by variations in experimental techniques.<sup>41,42</sup> Fidler has shown that the capacity to produce metastasis from human tumor cell lines of long duration<sup>43,44</sup> or of recent origin<sup>45-48</sup> depends both on the injection site and on the intrinsic properties of the cells.<sup>39</sup>

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). To study the properties of metastatic subpopulations from surgical specimens of human cancers, methods for their isolation, propagation, and testing must be developed.

The study of the biology of human renal cell carcinoma (HRCC) has been facilitated by the development of relevant *in vivo* models. Orthotopic implantation of HRCC cells allows for the isolation of tumor cells capable of producing metastasis to the lymph nodes, lung, and other organs. Furthermore, this system allows for analysis of the properties that identify metastatic HRCC under *in vivo* conditions.

## ESTABLISHMENT OF RENAL CANCER CELL LINES

We have used two HRCC cell lines, the SN12 and KG-2, to study the metastatic potential of HRCC. Naito and associates established the SN12 renal cell carcinoma line from a radical nephrectomy specimen of a renal cell carcinoma in a 43-year-old man.<sup>49</sup> A cell suspension with high viability was divided into three aliquots. One was used to produce a tissue culture line designated SN12C. The second was injected subcutaneously in the lateral aspect of the anterior thoracic wall of several nude mice. Two months later, a subcutaneous tumor was excised and dissociated enzymatically. The cells were established in culture, and the line was designated SN12S1. The third aliquot was injected into the kidneys of nude mice. Several weeks later, a large tumor was harvested. In one mouse, a grossly visible tumor nodule was found in the liver, and the peritoneum contained ascitic fluid. Tumor cells isolated from the kidney tumor and the liver nodule were established as individual cell lines in culture and designated lines SN12K1 and SN12L1, respectively. The cell line established from the ascitic cells was designated SN12A1.

The cells of the HRCC lines grew on plastic as monolayer cultures with similar morphologic features. The human origin of all five cell lines was ascertained by detailed karyotypic analysis and isoenzyme determinations. No contamination with mouse cells was found in any of the lines.

Similarly, the KG-2 cell line was isolated from a surgical specimen subsequent to radical nephrectomy in a 58-year-old man. Cells were injected into the subcutis, kidney, cecal wall, and spleen of nude mice. Tumor grew in the subcutis and kidney, and only kidney tumors produced distant metastases.

## BIOLOGIC BEHAVIOR OF THE DIFFERENT HRCC LINES

Subsequent experiments showed that the five HRCC SN12 cell lines consisted of cells with different biologic properties, including growth *in vivo* at ectopic and orthotopic sites and production of experimental and spontaneous metastasis (Table 2-1).<sup>49</sup>

The *in vitro* tumor cell doubling time did not differ significantly among the cell lines. All lines were tumorigenic at subcutaneous sites, but SN12L1 cells (developed from a metastasis) grew at the fastest rate, whereas SN12A1 cells (ascites) were the

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