

Clonal Selection of Metastasis within the Life History of a Tumor

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Introduction

Tumor metastasis is a clonally selective process that occurs as part of tumor progression, a hypothesis supported by experimental and clinical research. Data supporting this hypothesis were first introduced by Fidler and Kripke in the late 1970s (1), providing a powerful model to gain insight into the mechanism(s) of metastasis. Studies of cellular genetic and epigenetic heterogeneity within tumors (1–3) support the selective process of metastasis, as have studies of clonal selection and cellular expansion following metastasis (4, 5). In the original clonal selection studies (1), orthotopic tumor models were used because they faithfully reproduce clinical invasion, histopathology, cellular shedding, and metastasis (6), showing the clonal origin (1, 4) and selective mechanisms (7, 8) of metastasis.

Clonal Selection in the Process of Metastasis

The growth of a tumor focus at a site distant to the primary tumor represents the final step in the multistep process of metastasis, which has the potential to incorporate numerous selective events. Implicit to the *clonal selection* hypothesis (Fig. 1) is the presence within the primary tumor of at least one tumor population that expresses all of the up-regulated or inactivated genes required to successfully complete the metastatic process. As such, primary tumors may also have cellular subpopulations that express none or some of the characteristics required to complete the metastatic process. A corollary to the clonal selection hypothesis is the suggestion that individual metastases can originate from multiple clonal subpopulations that may have occurred within the primary tumor, resulting in biological diversity among metastatic foci. Thus, primary tumors can have multiple clonal subpopulations capable of forming metastatic foci, resulting in individual metastases expressing different phenotypes (5, 7). Further, due to the genetic instability (9), a clonal metastasis can rapidly become heterogeneous (5, 10). Although the clonal selection hypothesis was formally shown within rodent models in 1977 (1), it is confirmed daily by clinical pathologists. Histologic examination of primary tumors shows cellular heterogeneity based on morphology and expression of membrane receptors. For example, morphologically diverse areas can occur within a tumor such as foci of ductal carcinoma *in situ* within a primary breast carcinoma. Similarly, estrogen receptor, Her2/neu, or p53 expression varies between and within tumors and metastases, ranging from 1% to >90% of cells. Thus, clonal selection of metastases can result in phenotypically diverse metastases (intra-host heterogeneity), such that within the same patient

some metastases can be positive whereas others are negative for a specific phenotype.

Clonal selection and intra-host metastatic heterogeneity have strongly been supported by murine studies (4, 5, 7). However, clinical support has been less extensive due to experimental and methodologic challenges. Metastatic tissue may be obtained asynchronously, relative to primary tumor tissue, such that comparisons are complicated by tissue availability. Optimally, tissue samples are obtained from untreated patients and primary tumor tissue is compared with tissues from metastases at a single site because there are organ-specific genotypes. Several studies have successfully compared primary tumors to metastatic foci within the same patient based on a number of different phenotypes. Kuukasjarve et al. (11) analyzed the genetic composition of 29 primary breast carcinomas and paired asynchronous metastases by comparative genomic hybridization and fluorescence *in situ* hybridization (FISH). They found that 69% of the metastases had a high degree of clonality with the corresponding primary tumor, whereas chromosomal X inactivation patterns supported the remaining metastatic lesions as originating from the same clone as the primary tumor. It was concluded that although all metastases are derived from the parent tumor, metastasis could occur at various times, resulting in comparative genomic hybridization heterogeneity. Similar conclusions were derived by Gancberg et al. (12) who examined pairs of primary and metastatic tumors from 100 breast cancer patients and reported that 6% had discordant Her2/neu overexpression by the metastatic tissue as compared with the primary tumor. FISH analysis from 68 of the patients revealed a 7% difference between primary and secondary lesions, although these patients differed from those patients with differences in immunohistochemistry. In addition, they examined Her2/neu overexpression by multiple metastatic lesions from 17 patients, revealing that 18% differed in Her2/neu expression. Suzuki and Tarin (13) also examined matched, primary breast tumors and their metastases and concluded that there were limited but statistically significant differences between the primary tumors and their lymphatic metastases. In the study by Suzuki and Tarin (13), it was suggested that the genetic program for metastasis is developed over time, although occasionally it occurs early during tumor progression, ultimately resulting in heterogeneity both within and between metastases. Their results from detailed microarray and computational analyses revealed that a small number of genes were differentially expressed between tumors and metastases, supporting the conclusion that although metastases generally resemble the primary tumor on a mutational basis, a few genes differed consistently and seem to be of significant mechanistic importance. Together with the results by Urquidí et al. (14), these studies provide direct proof that individual cancer cells coexist within a tumor, differing in their metastatic capability such that metastatic primary tumors contain tumor subpopulations with widely variant metastatic proclivities and expression profiles. Montel et al. (15) also showed that as the metastatic proclivity of a tumor increases, the cellular populations within the tumor develop increasingly variable expression profiles.

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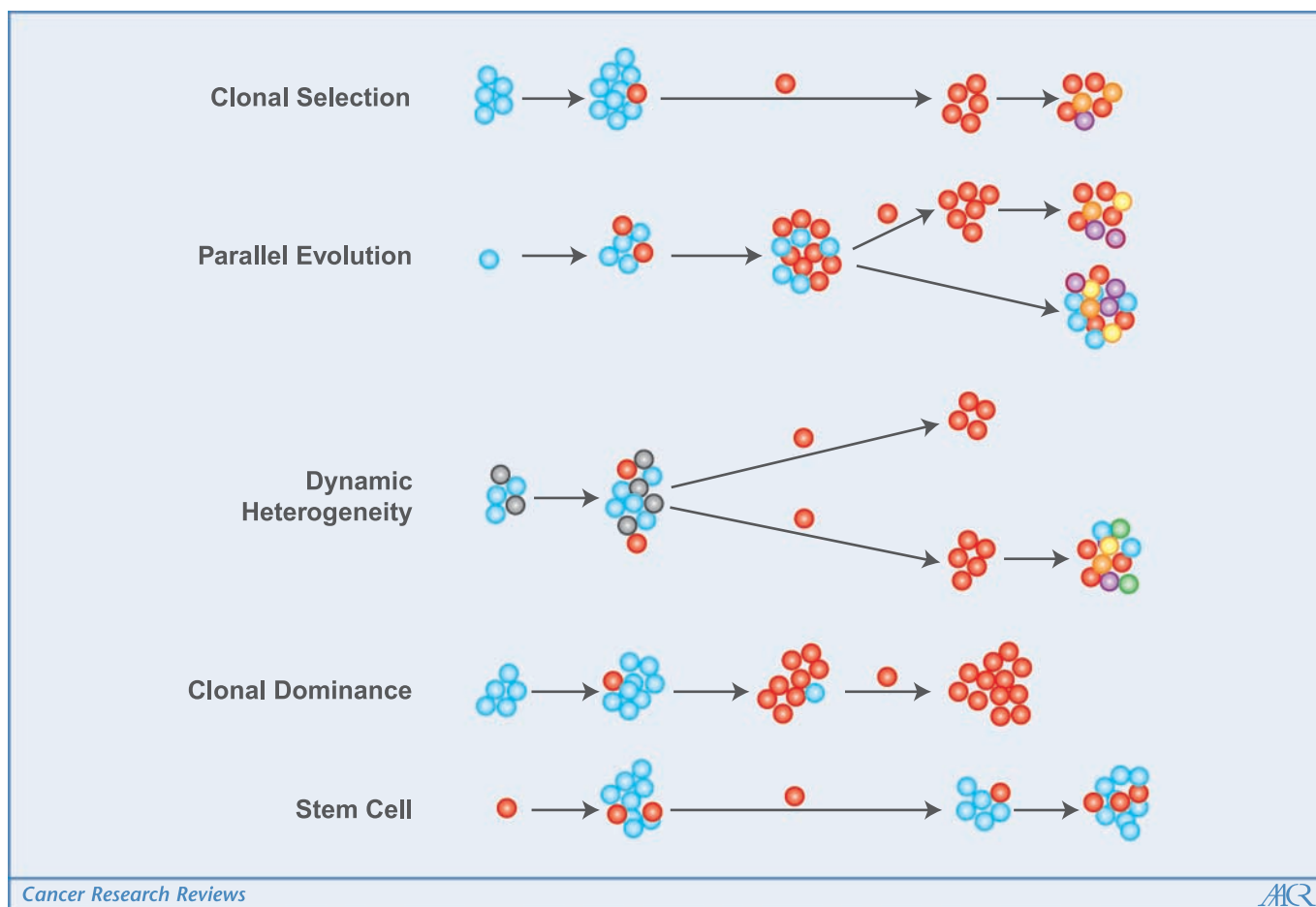


Figure 1. Models of the metastatic process. The *clonal selection* model of metastasis suggests that cell populations with all of the prerequisites for metastatic capacity are the subpopulations that metastasize. In the *parallel evolution* model, it is suggested that metastasis occurs early in tumor progression and independent of tumor cells at the primary site. The *dynamic heterogeneity* model suggests that the frequency at which metastatic variants arise within the primary tumor determines its metastatic potential. Further, this model has been suggested to allow cells within the metastatic foci to lose their metastatic properties. The model of *clonal dominance* suggests that metastatic subclones within a primary tumor outgrow the primary tumor and dominate the tumor mass as well as the metastases. In the *stem cell* model, it is suggested that only the stem cells, and not the bulk of the cells within the tumor, have the ability to metastasize and form new tumors.

Tumor progression (2) suggests that metastatic cells are exclusively found within metastases, but not necessarily within primary tumors. Further, tumor progression is a continuum and does not end with metastasis; it continues during therapeutic intervention such that variants develop and are selected for resistance to chemotherapy. Thus, tumor cells within a metastasis initially express all of the alterations necessary for metastasis; however, as metastatic foci grow, heterogeneity again develops, potentially including cells with different metastatic capabilities.

Alternative Hypotheses of Metastasis

In the decades since the clonal selection model was proposed, other metastatic models have been suggested. However, when considered within the life history of a tumor, many of these hypotheses seem to be consistent with the clonal selection hypothesis. In the *parallel evolution* model (Fig. 1), it is suggested that metastasis occurs early in tumor progression followed by a parallel evolution of primary and metastatic tumors (16). This hypothesis was initially based on a study by Schmidt-Kittler et al. (17) that found genetic alterations of breast cancer cells within the bone marrow that did not have the same phenotype as tumor cells

within the primary tumor. This hypothesis was extended by the suggestion that the genetic alterations that occur early in tumor progression confer a selective proliferative advantage as well as a metastatic propensity (18). The parallel evolution hypothesis is supported by microarray studies of primary tumors and the identification of expression profiles that may predict the risk of developing a metastasis (16, 17, 19). Nonetheless, as both oncologist and pathologist will attest, metastasis occurs before diagnosis, often years earlier. Further, tumor cell dissemination is but one step in the metastatic process and tumor cells can be found in the marrow or peripheral blood of patients who do not have and indeed never will develop a gross metastatic lesion. Thus, the lack of a metastatic microarray profile by a single cell from the marrow is expected (17) because cells that disseminate need not predict for, and indeed differ from, cells that can develop into gross metastatic foci.

Molecular signatures have been suggested, which provide a distinction between tumors that will relapse and those that can be cured surgically. Unfortunately, the prediction rates from prognostic microarrays may be no better than conventional prognostic paradigms (20). Furthermore, a microarray analysis of a primary tumor or a metastasis reflects the entire population of cells within the lesion and cannot reflect the frequency of cells that express all

of the genetic attributes required to form a metastasis (21). Thus, a microarray analysis may not test the hypothesis of parallel evolution because one must examine individual cells within the primary tumor or metastasis to discover whether they express all of the genetic attributes required for metastasis (21). Kang et al. (22) addressed this concept, in part, with a microarray analysis of cellular clones within tumors, resulting in a demonstration of the multigenic nature of metastasis (23). They also showed that some, but not all, of the cells in a primary tumor express the genetic subset required to metastasize. These studies support the clonal selection hypothesis and the concept that parallel evolution is expected from tumors experiencing the strong selective pressures associated with therapeutic intervention. Thus, if one accepts the clonal original of tumors and metastases, then some homogeneity between the cells in the primary tumor sites and metastatic foci (24) is expected despite genetic instability.

The *dynamic heterogeneity* model (Fig. 1) of metastasis suggests that the frequency of metastatic variants within a tumor determines metastatic potential (25). Further, metastatic variants may be unstable, resulting in a dynamic equilibrium between the generation and loss of cells with a metastatic phenotype (26). Thus, it has been

suggested the dynamic heterogeneity model incorporates the concept of a transient metastatic phenotype. However, studies of clonal variants from murine and human tumors have shown the stable expression of high and low metastatic populations by both experimental and spontaneous metastasis assays (3, 4, 8, 10). The dynamic heterogeneity model has also been suggested to incorporate epithelial-mesenchymal transitions (EMT). Malignant tumors commonly contain areas that are histologically described as anaplastic (i.e., tumors fields with rapidly dividing cancer cells, which appear as foci of EMT). EMT refers to a process whereby cells undergo a switch from an epithelial phenotype with tight junctions and lateral, apical, and basal membranes and a lack of mobility to mesenchymal cells with loose cellular interactions, a nonpolarized, motile phenotype, and an extracellular matrix. EMT was initially identified as an early step in embryology; however, recently, it was introduced as a potential mechanism of metastasis. EMT is invoked within the dynamic heterogeneity model such that metastasis is a transient process affecting only a small tumor population. However, the small areas of EMT may actually represent tumor and stromal cell interactions, which can induce transitory, epigenetic/transcriptional changes, and a phenotypic shift by tumor cells. This may be

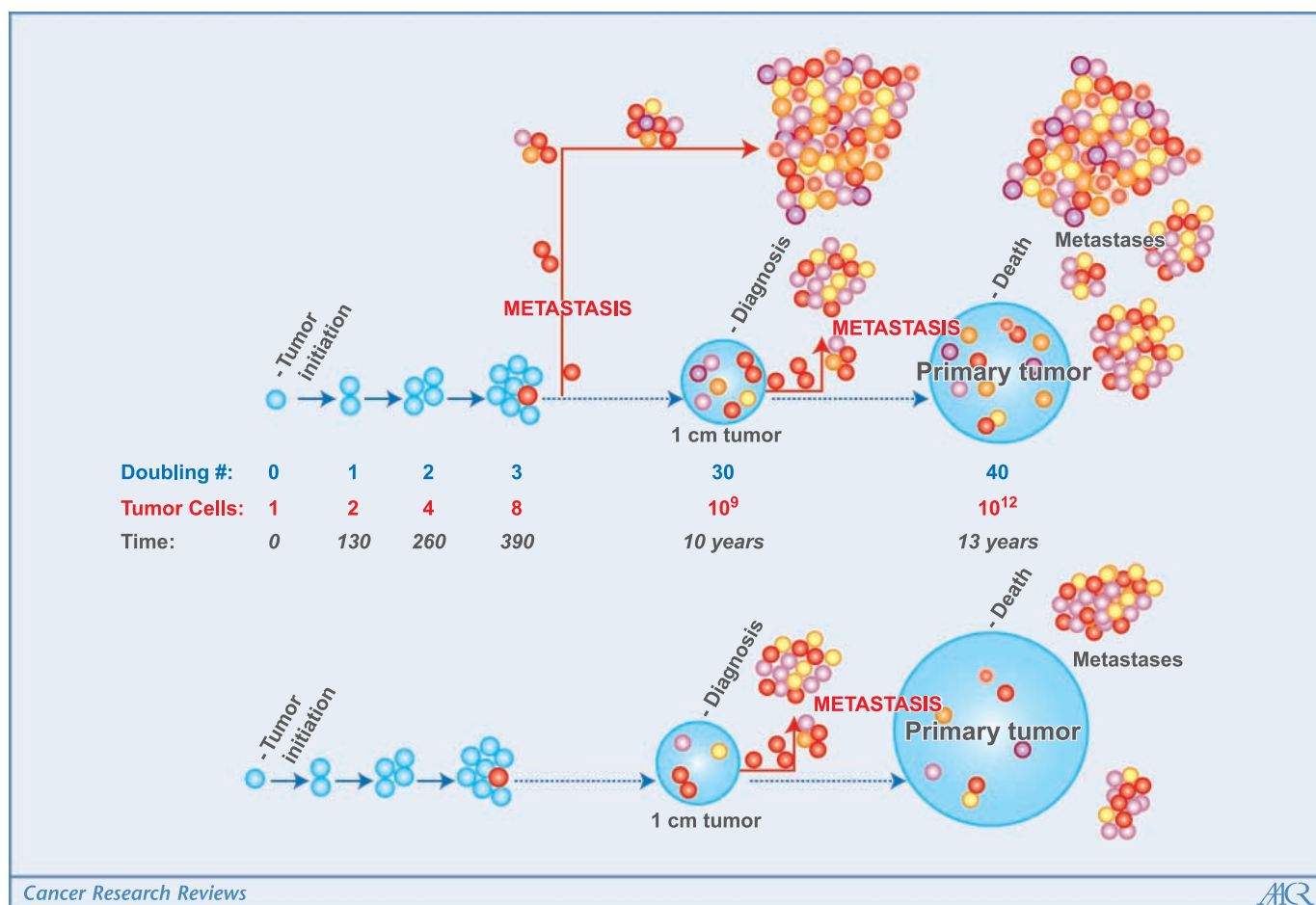


Figure 2. Overview of clonal selection within the life history of a tumor. During tumor initiation, progression, and metastasis, ~30 doubling times or an estimated 10 y is required to achieve the 1-cm tumor (10^9 tumor cells) needed for clinical diagnosis. During this time frame, genetic instability results in metastatic variants such that metastases occurring closer to the time of diagnosis are smaller and less heterogeneous as compared with ones that occur earlier. This is contrasted with tumors with less genetic instability, which develop metastases late in tumor progression, are small in size at diagnosis, and have less heterogeneity. Regardless of the timing of metastases, if a 1-cm tumor is left untreated, then a lethal tumor volume, which is $\sim 10^{12}$ tumor cells, occurs within 10 doubling times, which calculates to 3 y. The overall conclusion is that the time from tumor initiation to diagnosis represents a longer period in the life history of a tumor compared with the time during which we study a tumor post-diagnosis.

cell contact dependent, providing an epigenetic interaction critical to the metastatic process (27). However, the transcriptomic contribution of such a transient population, if analyzed as a microarray signature, is lost within the total tumor population. Stromal-epithelial interactions provide a rational explanation for EMT as there is little convincing evidence for the conversion of epithelial cells into mesenchymal cells *in vivo*. Further, the genotypic and phenotypic repertoires of malignant cells are sufficient to account for the events and processes believed to occur during EMT without requiring a radical change in cell identity or cell differentiation (28).

A similar hypothesis identified as the *clonal dominance* model (Fig. 1) suggests that once a metastatic subclone emerges within a primary tumor, cells from this subclone will outcompete and dominate the tumor mass itself, resulting in phenotypic similarities between the primary tumor and the metastatic foci (29). In addition, the *stem cell theory* of cancer (Fig. 1) suggests that tumors have rare cells with infinite growth potential (30). These cells may metastasize and some of the cells within the metastatic foci may then differentiate into cells without a metastatic phenotype. However, it is not clear whether tumor stem cells are "true" stem cells or represent a highly malignant cellular subpopulation.

Role of Tumor Life History in Metastasis

The clonal selection hypothesis, as well as other hypotheses of metastasis, must be considered within the context of the life history of a tumor and not just that portion monitored following diagnosis. The cellular doubling time of a carcinoma is between 44 and >1,800 days during exponential growth (31). In the following discussion, we will use variables from breast cancer patients whose primary tumors have a doubling time of ~130 days during exponential growth (32, 33). Unfortunately, tumor lesions smaller than 1 cm are rarely diagnosed due to the limitations of our routine diagnostic instruments. This is clinically significant as a 1-cm-diameter tumor has 10^9 cells and has undergone at least 30 doubling times (10 years at a 130-day doubling time) from tumor initiation to diagnosis. Based on these variables and the observation that a tumor burden of ~1,000 cm³ is generally lethal (34), we can calculate that the time from diagnosis to mortality represents only 10 doubling times for a 1-cm tumor. Thus, three quarters of the life history of a tumor has occurred by diagnosis, allowing the coalescence of hypotheses for mechanisms of metastasis.

In Fig. 2, we have overlaid the clonal selection hypothesis within the life history of a tumor. We posit that metastatic mechanisms, including clonal dominance, dynamic heterogeneity, and parallel evolution, represent subsets of the clonal selection hypothesis when viewed within the life history of a tumor. Clonal dominance is easily incorporated as primary tumors and metastatic foci are both subjected to strong selective pressures following diagnosis. Further, these selective pressures may remove variants with less competitive growth properties or which are sensitive to therapeutic intervention, thereby reducing cellular heterogeneity. However, cells with a metastatic phenotype have an increased genetic instability (9), allowing for the development of cell populations within a metastatic site that express both increased and decreased metastatic phenotypes. This has been shown within studies using cells from clonal origin, metastatic foci based on sensitivity to chemotherapeutic agents, as well as metastatic heterogeneity (5). Further, the cellular homogeneity induced by cloning results in increased genetic instability and restoration of genetic divergence

(35). The parallel evolution model can also be encompassed within the clonal selection model. Because of the long time period between tumor initiation and progression to a size that can be diagnosed, there is ample time for cellular divisions for cellular heterogeneity and survival. Thus, metastasis may occur years before tumor diagnosis, yet still have occurred late in the life history of a tumor, such that even if parallel evolution occurs late in the life history of a tumor, there remain years for heterogeneity and selection to occur.

There is also a consensus between the models of clonal selection and dynamic heterogeneity such that if a tumor has a high frequency of metastatic progenitors, then a high incidence of metastases might be expected compared with a tumor with a low frequency of metastatic progenitors. The dynamic heterogeneity model also states that metastasis occurs early relative to diagnosis such that within the life history of a tumor metastasis, it might have occurred a decade before diagnosis and remains dormant for still longer. Outcomes from considering this time frame include the potential for metastasis to occur at various times during tumor progression, resulting in metastases of different clonal origins, size (early or late in tumor growth), and extent of molecular and phenotypic heterogeneity. These characteristics vary dependent on when metastasis occurs within the life history of a primary tumor as well as on the unique attributes and genetic instability of the cellular clone. The dynamic heterogeneity model can also be studied using poorly metastatic versus highly metastatic tumors of clonal origin (5, 8). This maybe critical because tumors composed of cellular populations that are predominantly highly metastatic cannot support the selection of highly metastatic cells (3, 8). Equally, tumor clonality induced by either single-cell cloning or as an artifact of tumor transplantation (36) may also limit the analysis of cellular selection due to a lack of heterogeneity. In contrast, tumors composed of unselected, predominantly poorly metastatic tumor cells should support clonal selection. This has been shown using spontaneous metastases of clonal origin that rapidly become heterogeneous as assessed in assays of spontaneous and experimental metastases (5, 8). In these studies, it was shown that metastases of clonal origin rapidly became heterogeneous, potentially due to genetic instability, including clones with significantly lower metastatic properties than the parent metastases.

Summary

In summary, this Point-Counterpoint has focused on the hypothesis of clonal selection and the need to study a metastatic hypothesis within the life history of a tumor. Further, we stress the criticality of studying metastasis using suitable models, experimental strategies, and protocols. In addition, the metastatic process needs to be studied *in vivo* and within the context of host-tumor interactions and the life history of a tumor, as well as orthotopic, spontaneous metastases (6, 8). Despite the controversy about the clonal selection hypothesis, we hope we have shown that this powerful hypothesis remains strongly supported by data and is even more relevant to tumor biology when viewed in light of the life history of tumors.

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