Review

Mechanisms of metastasis

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Abstract

Metastasis is an enormously complex process that remains to be a major problem in the management of cancer. The fact that cancer patients might develop metastasis after years or even decades from diagnosis of the primary tumor makes the metastatic process even more complex. Over the years many hypotheses were developed to try to explain the inefficiency of the metastatic process, but none of these theories completely explains the current biological and clinical observations. In this review we summarize some of the proposed models that were developed in attempt to understand the mechanisms of tumor dissemination and colonization as well as metastatic progression.

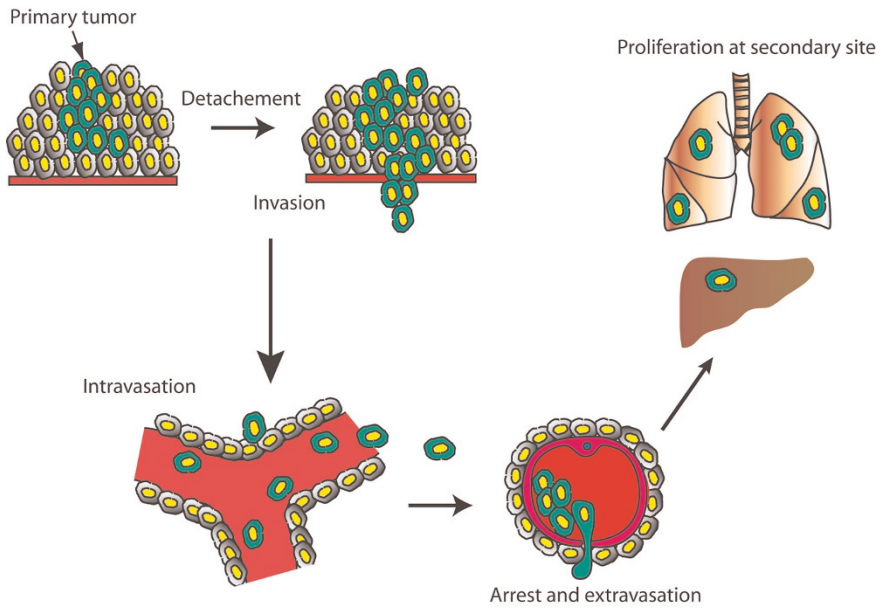
Metastasis is an extraordinarily complex process. To successfully colonize a secondary site a cancer cell must complete a sequential series of steps before it becomes a clinically detectable lesion. These steps typically include separation from the primary tumor, invasion through surrounding tissues and basement membranes, entry and survival in the circulation, lymphatics or peritoneal space and arrest in a distant target organ. These are usually, but not always [1], followed by extravasation into the surrounding tissue, survival in the foreign microenvironment, proliferation, and induction of angiogenesis, all the while evading apoptotic death or immunological response (Figure 1; reviewed in [2]).

Metastasis is of great importance to the clinical management of cancer since the majority of cancer mortality is associated with disseminated disease rather than the primary tumor [2]. In most cases cancer patients with localized tumors have significantly better prognoses than those with disseminated tumors. Recent evidence suggests that the first stages of metastasis can be an early event [3] and that 60% to 70% of patients have initiated the metastatic process by the time of diagnosis. Therefore, an improved understanding of the factors leading to tumor dissemination is of vital importance. However, even patients that have no evidence of tumor dissemination at presentation are at risk for metastatic disease. Approximately one-third of women who are sentinel lymph node negative at the time of surgical resection of the primary breast tumor will subsequently develop clinically detectable secondary tumors [4]. Even patients with small primary tumors and node negative status (T1N0) at surgery have a significant (15% to 25%) chance of developing distant metastases [5].

In spite of the prevalence of secondary tumors in cancer patients, metastasis is an extremely inefficient process. To successfully colonize a distant site, a cancer cell must complete all of the steps of the cascade. Failure to complete any one step results in failure to colonize and proliferate in the distant organ. As a result, tumors can shed millions of cells into the bloodstream daily [6], yet very few clinically relevant metastases are formed [7]. Although many steps in the metastatic process are thought to contribute to metastatic inefficiency, our incomplete understanding of this process suggests that we are aware of some but not all of the key regulatory points. For instance, destruction of intravasated cells by hemodynamic forces and sheering has been thought to be a major source of metastatic inefficiency [8]. However, recent evidence suggests that this may not always be the case and that cells in the bloodstream have been shown to arrest in capillary beds and extravasate with high efficiency and reside dormant in the secondary sites for long periods of time [9], sometimes for years [10]. Micrometastases may form, but the bulk of these pre-clinical lesions appear to regress [9], probably due to apoptosis [11].

It is apparent, therefore, that a comprehensive understanding of the biological and pathological intricacies underpinning the process of metastasis is still lacking. This is due, in part, to the sheer complexity of the metastatic cascade, which encompasses not only the biology of the tumor cell but also the rest of the organism in which it resides. Many models have been developed to attempt to provide a working hypothesis upon which to base further research. Here we review a number of the commonly accepted or recently proposed models and mechanisms of metastatic progression. We believe that many of these models are, however, somewhat inconsistent with current biological observation and that none sufficiently explain all of the complexities

Figure 1



The metastatic process. The initial steps of metastasis require proliferation of the primary tumor and invasion through adjacent tissues and basement membranes. This process continues until the tumor invades blood vessels or lymphatic channels, when individual tumor cells detach from the primary tumor mass and are carried via the blood or lymph to a distant target organ. Subsequently, tumor cells arrest in small vessels within the distant organ, extravasate into the surrounding tissue and proliferate at the secondary site. All of these steps must be performed while tumor cells avoid and survive apoptotic signals and host immune responses.

associated with this process. In spite of this, valuable insights have been gleaned from many of them, which have helped to further our understanding of the underlying mechanisms of tumor dissemination and colonization.

The progression model

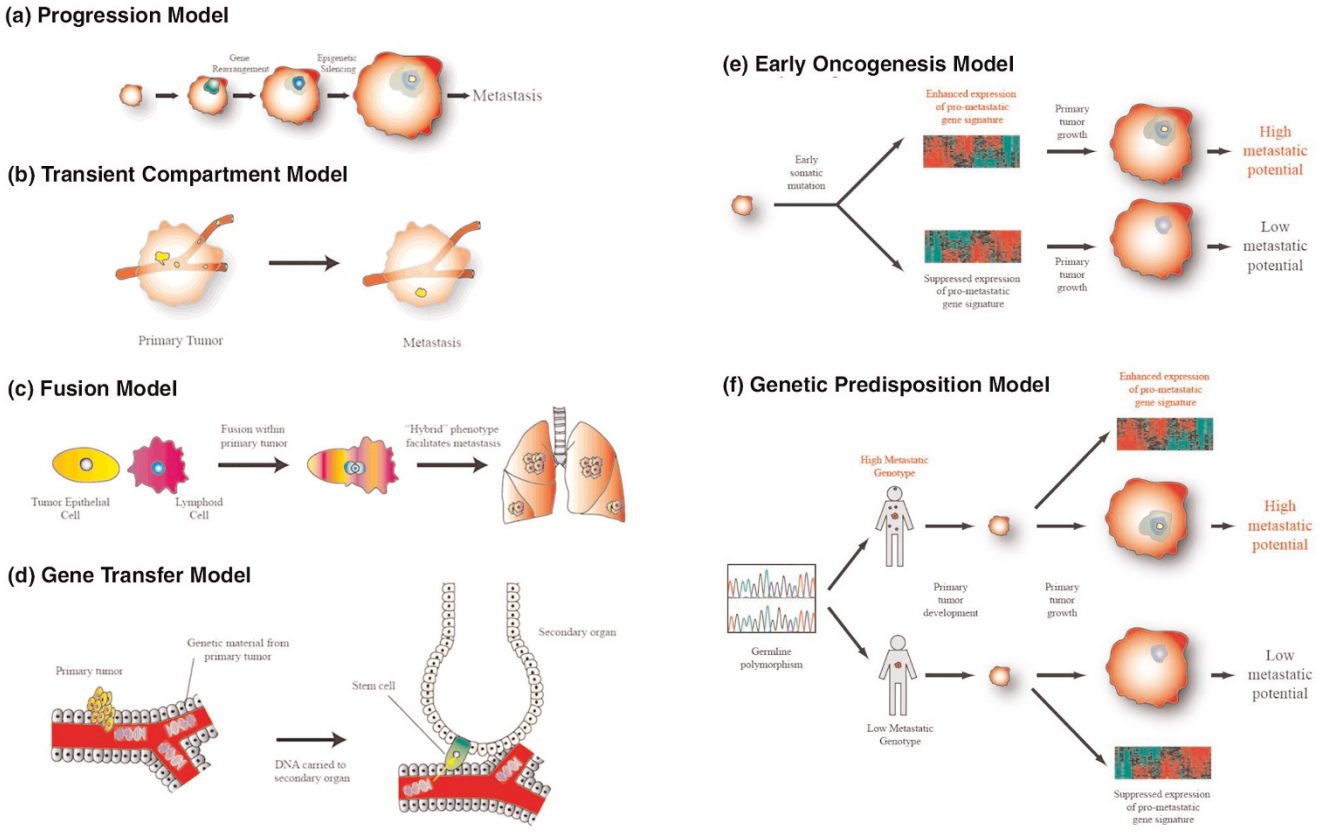
The most commonly accepted model of metastasis for the past 30 years is the progression model (Figure 2a). Originally proposed by Nowell [12], this model suggests that series of mutational events occur either in subpopulations of the primary tumor or disseminated cells, resulting in a small fraction of cells that acquire full metastatic potential. The inefficiency of metastasis is explained in this model by the low probability that any given cell within the primary tumor will acquire all of the multiple alterations required for the successful implementation of the metastatic cascade.

A number of studies over the years have provided support for this model. Clonal derivatives of cell lines have been demonstrated to have different metastatic capacities [13], indicating that metastatic subpopulations do exist, at least in *in vitro* systems. More recently, similar studies have been performed to demonstrate that target organ tropism also results from genomic alterations within cell populations. Using a related cloning and selection strategy in a human tumor-derived cell line, Massague and colleagues [14-16] have demonstrated that distinct sub-populations of cells exist that have acquired specific gene expression patterns that predispose them to metastasize to particular organs, presumably by some form of somatic alteration.

The existence of metastasis suppressor genes also supports the hypothesis that somatic events are important intermediaries in metastatic progression. Metastasis suppressors are genes that when expressed in metastatic cell lines suppress the ability to form macroscopic metastases while having little or no impact on primary tumor growth [17,18]. Down-regulation of metastasis suppressors in tumors has been commonly associated with either loss of heterozygosity (for example, [19]) or transcriptional silencing [20], with mutational inactivation rarely being observed.

In spite of the accumulation of evidence supporting this model, however, paradoxes remain, one of which is the existence of patients with unknown-primary cancer metastatic disease. These patients, which constitute approximately 5% of solid tumor-related cases, typically present with disseminated disease but have no clinically detectable primary tumor or only a small, well differentiated lesion that is found at autopsy [10]. The stochastically driven progression model suggests that a primary tumor must possess a sufficient number of cells to achieve the necessary sequence

Figure 2



A number of models have been proposed to explain the biological complexities of metastasis. **(a)** Progression model. A primary neoplasm gains a progressively more metastatic phenotype through a stochastic accumulation of somatic mutations. **(b)** Transient compartment model. All viable cells in a tumor acquire metastatic capacity, but due to positional and/or random epigenetic events only a small fraction are capable of completing the process at a given moment in time. **(c)** Fusion model. To gain a fully metastatic phenotype, a tumor cell must acquire certain characteristics of lymphoid cells (for example, proteolytic degradation, the ability to intra- and extravasate). This phenotype is achieved by nuclear transduction with cells of myeloid origin. **(d)** Gene transfer model. A characteristic of malignancy is the presence of tumor DNA in the bloodstream. This DNA, carrying the somatic mutations associated with neoplasia, is carried to the secondary site. Subsequently, the tumor DNA is absorbed by stem cells at the distant organ, which endow the stem cell with malignant properties. **(e)** Early oncogenesis model. The metastatic potential of any primary tumor is set early in its evolution, presumably as a consequence of somatic mutation. This is why it is possible to accurately predict prognosis from bulk tumor tissue using microarray gene expression signatures. **(f)** Genetic predisposition model. The metastatic potential of any primary tumor is altered by the genetic background upon which it arises. That is, an individual will be more or less susceptible to tumor dissemination as a consequence of constitutional polymorphism. Such germline variations influence all aspects of the metastatic cascade, including the expression of pro-metastatic gene expression signatures within the primary tumor.

of events that lead to metastasis. The absence of large primary tumors in individuals with unknown-primary cancer metastatic disease therefore runs contrary to this model. A second paradox is the finding that although variant clones with high metastatic capacity can be identified in populations, it is frequently observed that these variants revert to a low-metastatic capacity after several generations [21,22]. Permanent somatic events that induce metastatic capacity, as predicted by the progression hypothesis, would be expected to be stably inherited, rather then being rapidly lost. Therefore, due to the inherent inconsistencies and paradoxes of the progression model an alternative explanation termed 'dynamic heterogeneity' has been proposed.

The transient compartment models

The dynamic heterogeneity model [22], and the subsequently extended transient metastatic compartment model of Weiss [23] were proposed to explain the lack of consistent increases in metastatic capacity of secondary tumors compared to primary tumors. If metastatic capacity was due to a series of heritable mutation events, as predicted in the progression theory, it might be expected that cells that had successfully completed the metastatic cascade would be more efficient at establishing new metastatic tumors than the primary tumor. However, this was not consistently observed in a number of experimental systems (reviewed in [23]). The transient metastatic compartment model proposed by Weiss

suggests instead that all viable cells in a tumor acquire metastatic capacity, but due to positional and/or random epigenetic events only a small fraction are capable of completing the process at a given moment in time (Figure 2b) [23]. Thus, although a tumor may have been derived from a cell that successfully completed the metastatic process, not all cells within such a tumor retain the capacity to colonize secondary sites due to random, or microenviromentally induced epigenetic events or inadequate access to vasculature.

Support for this model comes from studies demonstrating that methylation inhibitors can modulate the metastatic capacity of cell lines [24-28]. However, while global demethylation may mimic some of the proposed epigenetic events, these agents can cause chromosomal aberrations [29], opening up the possibility that the modulation of metastatic capacity was due to mutational rather than epigenetic events. In addition, genomic instability is a hallmark of solid tumors, and increases in the number of chromosomal aberrations often correlate with a poorer prognosis [30]. The inability of cells isolated from metastases to be consistently more metastatic than the primary tumor could be explained by additional genomic events within cells that disrupt the delicate balance of molecular processes required to successfully complete the metastatic cascade [31]. Furthermore, the transient compartment model does not explain the clonal nature of metastases [32-34]. Since neoplastic cells within primary tumors are known to be heterogeneous [35], if every cell had a metastatic ability that was modulated only by transient epigenetic events, then it is less likely that significant proportions of secondary tumors would appear to be of clonal origin [13,36,37].

The fusion model

Tumor cells must acquire a number of different characteristics beyond unlimited proliferative capacity to become metastatic. These include detachment from the basement membrane, loss of gap junction [38,39] and tight junction contacts [40] with neighboring cells, migration away from the primary tumor site, through extracellular matrix [41] either via proteolytic mesenchymal-like or proteolytic-independent amoeboid motion [42], entrance and survival in the vasculature or lymphatics before arresting in the secondary site and proliferating either within the vessel [1] or after extravasation [9] into the surrounding parenchyma. How tumor cells acquire all of these abilities is clearly of great interest. The progression model suggests that dedifferentiation as a result of accumulation of somatic mutations produces a more embryonic phenotype. Intriguingly, while these abilities are foreign to the epithelial cells that form the bulk of human solid tumors, they are characteristic of certain cells originating from lymphoid tissues. This has led to several alternative explanations as to why tumor-derived epithelial cells gain the ability to metastasize, all of which center around the hypothesis that metastatic tumor cells acquire lymphoid characteristics. The presumed origin of the lymphoid characteristics of these epithelial cells is by nuclear transduction, either by fusion with cells of myeloid [43] origin or by uptake of tumor DNA present within the circulation [44] (Figure 2c,d).

Cellular fusion as a source of genomic instability in cancer is an idea that developed originally from studies of fertilization. It was observed in the 1800s that eggs experimentally fertilized with multiple spermatozoa underwent abnormal mitosis, which suggested that similar chromosomal imbalance might result in oncogenesis (reviewed in [45]). Further work by Aichel and Mekler proposed leukocytes as a potential fusion partner, and that the subsequent acquisition of leukocytic characteristics by tumor epithelial cells could be a prerequisite for the development of a more metastatic phenotype [45].

Cellular fusion is unequivocally an important part of normal human physiology. Myocytes fuse to form multinucleate skeletal muscle fibers [46]. Osteoclasts, foreign body giant cells and Langhan's giant cells are thought to be formed by fusion of cells of the monocyte/macrophage lineage [47]. Tumors can be significantly populated with macrophages, with the degree of macrophage infiltration being associated with disease outcome (reviewed in [48]). The presence of high numbers of cells with leukocytic, phagocytic and fusogenic properties in tumors has led a number of investigators to suggest that they may be tumor cell fusion partners, and that it is these cells that endow tumor epithelial cells with many of the characteristics necessary to disseminate and colonize distant sites [49-52].

Early evidence supporting this hypothesis includes a number of experimental transplant systems. In 1974, Goldenberg *et al*. [53] reported that transplants of an astrocytic glioma into the cheek pouches of nine immuno-competent hamsters resulted in the outgrowth of a single metastatic tumor that could be serially passaged. Karyotyping of tumor cells revealed the presence of both human and hamster chromosomes, indicating that cellular fusion had occurred. However, analysis of tumor cells in the fifth ascites and fifteenth cheek pouch passage showed massive loss of human chromosomes. These data, plus the previous demonstration that rodent-human hybrids segregate human chromosomes [54] suggest that the glioma did not acquire metastatic capacity by fusion, rather that a metastatic hamster tumor resulted from the aneuploidy induced by fusion with the human tumor.

Other more convincing evidence of *in vivo* fusion of tumor and host cells does exist. For example, a number of groups have evidence that fusion can occur between endogenous host cells and transplanted tumor cell lines through the use of syngenic cell lines (for example, [55-58]). However, some of these cell lines were known to be prone to undergoing fusion

in vitro, which might have impacted the relevance of these observations to the autochthonous setting. This also raises the question that the hybrids observed in these experiments might be due to artifacts of tissue culture. Contamination with uncharacterized viruses is only one of several caveats that needs to be considered when evaluating these data [59].

Evidence for spontaneous cellular fusion in solid tumors in humans does exist, however. Two cases of renal carcinoma in bone marrow transplant recipients have been described in which the tumor cells bear markers of both the bone marrow donor and the recipient [60,61]. Fusion events similar to these would clearly have deleterious effects on the cell, but whether oncogenesis was initiated by cellular fusion or was merely a secondary event that occurred later during tumor growth and evolution is unclear. In addition, whether or not the treatment associated with bone marrow transplantation contributed to the fusion event also has to be considered. A recent study, however, has demonstrated that fusion can occur between an established tumor cell and a normal cell in human neoplasia. Specifically, examination of osteoclast nuclei in myeloma patients demonstrated the presence of myeloma-specific chromosomal translocations [62]. Furthermore, it was postulated that this type of fusion event might contribute to the bone destruction that is a prominent characteristic of this disease.

Whether or not cell fusion occurs frequently in cancer patients and contributes to metastatic progression is still an open question. *In vitro* fusion of cells can lead to subclones with varying metastatic potentials (for example, [51,55,63]). However, metastatic capacity is known to vary within cell lines, as demonstrated by Luria-Delbruck fluctuation analysis [64]. Thus, it is unclear whether the changes in metastatic ability of such hybrids truly represent properties acquired due to fusion or represent stochastic subcloning and selection events, particularly when some of the cell lines used in these experiments were originally derived from metastatic tumors [65].

Thus, at the present time, there does not appear to be conclusive evidence that cellular fusion plays a significant role in the acquisition of metastatic capacity, which could possibly reflect the fact that it is not a mechanism of metastatic dissemination and growth. Conversely, it may well be indicative of the fact that identifying fusion events of two cells from the same individual in highly aneuploid solid tumors is a dauntingly difficult task. So while acquisition of leukocytic traits by monocyte fusion remains an interesting and potentially attractive hypothesis, it has not gained a significant following in the cancer research community to date.

Gene transfer models

A second, related hypothesis for the acquisition of metastatic capacity is that of horizontal gene transfer. This theory is basically a resurrection of a debate among nineteenth century physicians on whether unknown substances released by primary tumors were responsible for neoplastic conversion of normal cells in secondary sites, or whether cell trafficking of malignant tumor cells occurred. Improved microscopy and histological technology eventually demonstrated that cellular escape of malignant cells from the primary tumor did occur [66], which ultimately resulted in Paget's seed-soil postulate in 1889 [67]. The idea that metastatic capacity could be induced by horizontal transfer of tumor phenotypes was resuscitated many years later, after it was recognized that circulating tumor DNA was present in animal tumor models [68] and cancer patients [69]. This theory has been revived again in recent years as the genometastasis hypothesis [44], which is based on the observation that, in some circumstances, horizontal gene transfer has been observed in experimental systems (for example, [70]). Specifically, it has been suggested that metastases arise not from circulating cells, but instead from *in vivo* uptake of circulating DNA by stem cells at the secondary sites [71]. Thus, metastasis would not be the progeny of primary tumors, but instead *de novo* tumors arising in cancer patients.

A number of observations have limited enthusiasm for this hypothesis. First, this hypothesis does not explain the organ specific tropism of metastases. It has been known for almost 120 years that cancers exhibit specific target organs for dissemination and colonization [67]. Since there is no reason to suspect that circulating DNA does not reach all tissues of the body, the genometastasis hypothesis would require tissue specific uptake or expression of the oncogenic DNA. While this is formally possible, at present there is no evidence for this phenomenon *in vivo*. Second, the *in vivo* transfection event would have to result in sufficient uptake of primary tumor DNA to re-program cells at the secondary site so that they consistently resemble the morphology of the primary tumor, as is usually observed [66,72,73], rather than a *de novo* tumor in the target organ. Third, sufficient DNA uptake would have to occur so that the resulting 'metastases' consistently express the molecular markers of the primary tumor organ in the secondary site [74]. The probability of the reproducible occurrence of these events, particularly in cases where large numbers of metastases exist within a patient, is low. Thus, although circulating DNA may be present in patients, logic would seem to mitigate against this being a common mechanism for induction of secondary tumors.

Early oncogenesis models

The application of microarray technology to the problem of metastasis has recently led to a variation of the transient compartment model termed the 'early oncogenesis model'. In 2002 to 2003, two different groups found that by using microarrays to quantify global gene expression patterns in bulk human tumor tissue, it was possible to identify gene signature profiles that can distinguish metastatic and non-metastatic tumors [75,76]. Since that time, other studies have reported similar findings [77-80].

These results led to a re-examination of the progression model, which states that only a small subpopulation of primary tumor cells will acquire the complete phenotype necessary to successfully colonize distant organs. However, the microarray gene signature expressed by tumors more prone to dissemination is present in bulk tumor tissue, suggesting that the majority of cells within a primary tumor must possess an inherent metastatic capacity. Therefore, a pro-metastatic gene signature expressed within a small subpopulation of cells within a primary tumor, as would be predicted by the progression hypothesis, would be masked by the larger bulk of the tumor.

As a result, several groups have proposed that metastatic propensity is established early in oncogenesis, potentially even by the same sets of activation/inactivation events that result in the primary tumor, an hypothesis that runs contrary to the somatic evolution model [76,81] (Figure 2e). The early establishment of the metastatic state would result in the bulk of the tumor cells harboring the metastatic gene expression signature. In addition, this hypothesis might explain metastatic disease of unknown primary origin. If the same oncogenic events drove metastasis, then it can easily be imagined how small tumors might immediately begin dissemination and colonization of distant sites.

As with the other models of metastatic progression, caveats exist with this model as well. First, if metastatic behavior is primarily determined by early oncogenic events, then one would predict that the majority of tumor epithelial cells would possess the ability to metastasize, and thus the efficiency of colonization of distant organs would be much higher than observed in clinical practice. In addition, the microarray results do not necessarily rule out the possibility that rare cellular subpopulations exist within primary tumors. Since the gene expression patterns represent an average of all of the cells within the bulk tumor tissue, it is possible that different subpopulations display various components of the metastatic pattern, but only a fraction of the entire program. Finally, this hypothesis is based on the supposition that metastasis gene expression profiles are induced by somatic oncogenic events. It does not account for the other major source of genomic heterogeneity observed in human cancer patients, that of inherited polymorphism.

Genetic predisposition model

The study of how genomic diversity due to inherited polymorphisms affects metastatic progression has been the major focus of our laboratory. Using a highly malignant mammary tumor transgenic mouse model, we demonstrated that the genetic background upon which the tumor arose significantly affected the ability of that tumor to successfully colonize the lung [82]. Since all tumors were induced by the same oncogenic event, the activation of the transgene, these results suggest that inherited polymorphism is a significant factor in addition to whatever metastasis-promoting somatic events occur in the tumor. In addition, since constitutional polymorphisms are present in all tissues of an individual, the effects of metastasis-promoting/suppressing polymorphisms may be apparent in tissues other than just the tumor epithelium. For example, it is possible that subtle variations in lymphocyte function due to germline-encoded differences in cellular function might have a significant impact on immunosurveillance. This, in turn, could result in a greater or lesser ability to clear disseminated tumor cells at the secondary site.

Following these initial observations, our laboratory has identified the first polymorphic metastasis efficiency gene, *Sipa1* [83]. An amino acid polymorphism was identified in mouse strains that reduced both the Rap1GAP activity of this molecule, as well as the ability of the Sipa1 protein to bind its cognate partner, Aqp2. The phenotypic effects of this polymorphism were modeled using RNA interference knockdown, which demonstrated that relatively minor reductions of Sipa1 levels significantly reduced the ability of a highly metastatic mammary tumor cell line to colonize the lung. These results were extended into human samples by performing pilot epidemiology studies, examining non-coding polymorphisms in the human ortholog. These studies demonstrated, as predicted by the mouse results, that polymorphisms in the human *SIPA1* gene were associated with markers of poor outcome in a Caucasian population-based breast cancer patient cohort [84], suggesting that *SIPA1*, and by extension other polymorphic genes, may play an important role in establishing metastatic susceptibility in humans as well as in mouse (Figure 2f).

These results have important implications that may be used to help resolve some of the paradoxes raised by other models. For example, the early oncogenesis hypothesis is based on the supposition that the prognostic gene expression profiles are due to somatic mutations. It does not take into account the fact that basal gene transcription rates can be significantly different in individuals of different genetic backgrounds. The ability of inherited polymorphism to influence gene transcription has been demonstrated in a number of different studies [85-87], and forms the basis of expression quantitative trait loci analysis. Analysis in our laboratory has demonstrated that at least some of the genes in one prognostic profile are, in fact, differentially expressed in high- versus low-metastatic genotypes [88]. Thus, it is possible that the metastasis predictive gene expression signatures are not just an indication of somatic mutations driving progression, but may also be a measure of inherited metastasis susceptibility segregating throughout the human population.

If this is true, then the progression and early oncogenesis models can be reconciled. Metastasis susceptibility, as reflected by the bulk tumor gene expression patterns, would be established early or before oncogenic transformation, as predicted by the early oncogenesis model. Subsequently, somatic mutations would occur as the tumor evolves until

subsets of cells acquire the necessary capabilities to complete the metastatic program, as predicted by the progression model. Importantly, the incorporation of genetic background into these models suggests that assessment of prognosis should be possible using non-tumor tissue, potentially even before cancer develops. If a sufficient fraction of metastasis risk is encoded by germline polymorphisms, rather than autonomous somatic events within the tumor, then any tissue in the body should carry some reflection of that risk. Gene products might be different between tissues, but since the underlying susceptibility polymorphisms are ubiquitous it would be theoretically possible to use any tissue to interrogate an individual's susceptibility state. As a proof of concept, our laboratory was able to show, at least in a mouse model, the ability to roughly define risk groups in mice using salivary gland protein profiles [88]. Whether this would be possible in human patients is currently unknown.

Effect of the microenvironment

An important implication of the effect of genetic background is the fact that it has an impact not only on the primary tumor, but all of the tissues of the body. This would also potentially play a role in establishment of the microenvironment of both primary and metastatic tumor cells. Investigation of the role of the microenvironment in metastasis has become of greater interest in recent years, and has been recognized as a potential major factor in progression. The role of immune cells and bone marrow are two of the areas of interest that have received recent focus. Work by the laboratories of Condeelis and Pollard have focused on the interaction of macrophages and tumor cells in the induction of tumor cell motility and vascular intravasation. Initial studies using the metastatic polyoma middle-T antigen transgenic mouse mammary tumor model [74] and a knockout of the *Csf1* gene demonstrated that depletion of macrophages did not significantly affect primary tumor formation, but resulted in a significant decrease in metastatic burden [89]. Further work by the same laboratories suggests that macrophages play an important role in inducing tumor cell motility at the primary site and may play an important role in the entry of tumor cells into the vasculature [90]. These findings are primarily based upon intervital video microscopy experiments that have revealed the frequent presence of macrophages at the sight of tumor cell penetration into capillaries. Most importantly, these results suggest that active participation of macrophages, and by extension other non-tumor cells, may be important obligate partners during the metastatic process.

Microenvironmental cues are thought to play important roles at the secondary site, as well as at the primary tumor. Recent studies by Lyden and colleagues have demonstrated that primary tumors induce the mobilization of bone marrow derived cells to the metastatic target organs, prior to the arrival of disseminated tumor cells [91]. These cells take residence within the target organ and are thought to establish a 'pre-metastatic niche', a term used to describe a set of novel microenvironmental stimuli that are conducive for tumor cell survival and growth [91]. Thus, the tumor cells both induce changes in the microenvironment by cytokine-mediated mobilization of bone marrow cells, and are subject to the novel conditions that the marrow-derived cells establish in the secondary site.

Conclusion

Metastasis, due to its complex spatial and temporal components, remains an enigma, despite all of our efforts to unravel its complexity. Many observations and hypotheses have been explored to explain the process, a number of which have been briefly described here. In our opinion, none of the proposed theories of metastatic progression can completely explain all of the observed clinical phenotypes. The different theories of metastasis are also not necessarily mutually exclusive. There is the real possibility that many of them are, at least in part, correct, and that there may be a number of different mechanisms by which a tumor cell may successfully colonize distant tissues. How these different potential mechanisms interact, intersect, or share commonality clearly should be a major focus of future research, since a clearer understanding of metastatic disease will be required to significantly reduce cancer morbidity and mortality. Fortunately for the field, new approaches, technologies, and most importantly, ideas are appearing within the literature and throughout different research laboratories. With these tools, hard work, and perhaps a little luck, we will be able to further unravel the inner workings of tumor progression and metastasis in the coming years and shed light on the mechanism(s) that drive the most lethal aspect of neoplastic growth.

Competing interests

The authors declare that they have no competing interests.

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