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Unravelling the complexity of metastasis — molecular understanding and targeted therapies

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Abstract

Despite recognizing the devastating consequences of metastasis, we are not yet able to effectively treat cancer that has spread to vital organs. The inherent complexity of genomic alterations in late-stage cancers, coupled with numerous heterotypic interactions that occur between tumour and stromal cells, represent fundamental challenges in our quest to understand and control metastatic disease. The incorporation of genomic and other systems level approaches, as well as technological breakthroughs in imaging and animal modelling, have galvanized the effort to overcome gaps in our understanding of metastasis. Future research carries with it the potential to translate the wealth of new knowledge and conceptual advances into effective targeted therapies.

Metastasis is the most deadly feature of cancer, accounting for greater than 90% of cancer-related mortality[1–3]. The clinical manifestation of metastatic lesions is the end result of a treacherous journey that few tumour cells are capable of completing, including local invasion and intravasation, survival in the circulation, homing and extravasation into the parenchyma of distant organs, and adaptation to the new environment and outgrowth of secondary lesions[1,3–5]. Although tumour cells that are shed from the primary tumour disseminate throughout the body, they tend to colonize select organs, with characteristically different periods of latency and efficiency depending on tumour type or subtype[1,5]. Steven Paget's century-old 'seed and soil' hypothesis likened tumour cells to 'seeds' that are systemically distributed, but that only inhabit particular environments, or 'soils', which are supportive to their sustained growth[6]. Despite the intellectual clarity of this hypothesis, understanding the exact molecular and cellular basis of the complex events that facilitate cancer metastasis has been difficult.

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We are now in an era in which systems level data are being generated at unprecedented rates, due, in large part, to a revolution in technology. Moreover, superior imaging modalities and experimental models afford the ability to explore the dynamic intricacies of cancer metastasis at a resolution that has not been achieved before (TIMELINE). We have begun to reveal fundamental concepts that underlie the development of metastasis: the lineage relationship between primary tumours and metastatic colonies[7–10]; the genetic properties of metastatic tumour cells that facilitate their interaction with the host stroma[11,12]; the unique functional contributions of different stromal components[11,13–16]; the organ-tropism of different cancer types and subtypes[17–20]; and the *in vivo* cellular and signalling dynamics during the different steps of metastasis[21–24]. With these newly acquired insights into the 'black box' of metastasis, the discovery of effective metastasis-targeting agents that specifically attack cancer cells and interrupt their communication with the microenvironment may soon be on the horizon. In this prospective Timeline article, we discuss possible new avenues for investigation that could lead to the development of novel approaches to prevent and to treat metastatic disease.

Characterizing metastatic seeds

Fundamental to understanding cancer progression is the identification of the distinguishing features that endow certain tumour cells with metastatic capabilities. Characterizing such features enables the discovery of potential diagnostic and prognostic biomarkers, as well as targeted therapeutic agents. Genomic profiling[25], second-generation sequencing[26], proteomics[27,28] and other systems level analytical techniques have dramatically accelerated the effort to comprehensively characterize metastatic tumour cells and to understand their natural history of evolution from primary tumours.

Molecular genealogy of metastasis.

It is generally agreed that cells acquire metastatic properties in the primary tumour, although there is an ongoing debate surrounding both the extent to which metastatic potential can be determined at this early stage in cancer progression and the lineage relationship between the primary tumour and metastatic lesions[29]. The linear and parallel models of metastatic progression are at the centre of this discussion (BOX 1). High-quality genomic studies have begun to explore the genetic and temporal relationship between primary lesions and metastatic lesions, the results of which will undoubtedly help us to resolve many important questions surrounding metastatic evolution[8,30].

DNA copy number analysis of metastatic prostate cancers has indicated a common clonal origin in most cases, although additional subclonal alterations were also observed in metastases[31]. Consistently, a comparison between whole-genome sequences of a basal-like primary breast tumour and its corresponding brain metastasis has shown that, although copy number alterations and overall mutational spectra within the genome were not significantly different, examining the prevalence of specific mutations revealed that a subset of cancer cells from the primary tumour were preferentially enriched in the metastatic lesion[9]. Genomic sequencing analyses of pancreatic cancer lesions also suggested that metastatic lesions are clonal in nature, and probably require additional driver mutations that are not

found in the primary tumour, and also that certain alterations are linked to organ-specific metastasis[32,33]. A recent study extended the metastasis genealogy investigation to the single-cell level, revealing that a single clonal expansion formed the bulk of the primary tumour and seeded the metastases[10]. Collectively, these studies suggest that metastases are spawned from a late clonal expansion of the primary tumour, and that such clonal cells may be additionally endowed with essential metastatic capabilities. However, we still have little grasp on exactly how long it takes for these metastatic clonal populations to emerge. Quantitative analysis of the mutation range in different stages of colorectal cancer suggested that the time required for an invasive tumour to develop metastasis (<2 years) is much shorter than the time needed for a benign tumour to evolve into advanced cancer[34]. In pancreatic cancer, the timing between these stages of cancer progression followed a comparable pattern, although it was shown that 5 years lapsed before a non-metastatic founder cell acquired metastatic abilities[33]. These findings imply that a fairly small number of rate-liming mutations are required for advanced tumours to gain metastatic competence.

Overall, these genomic studies seem to disagree with the parallel progression hypothesis that metastatic cells arise from early intermediate cells with genetic and phenotypic characteristics that differ from their primary tumour; they instead favour the linear progression model of metastasis[35]. A limitation of most of these studies is the use of cancer specimens from late-stage patients whose aggressive primary tumours and metastases were obtained within a relatively short time frame. Further investigations using retrospective or prospective samples that are collected in more diverse clinical scenarios, such as metastases that develop following prolonged latency, from large cohorts of patients are needed to provide a comprehensive view of the evolutionary dynamics of metastasis for different cancers. The insight we gain will guide the selection of treatment and will assist the design of preventive and therapeutic strategies.

Classification and prognosis.

An important task in cancer management is to identify early stage tumours that are at a high risk of metastatic spread. Gene expression profiling of breast cancer samples revealed the existence of distinct subtypes, which not only define the biological diversity of breast cancer[36], but which also engender meaningful prognostic and predictive value for patients[37–39]. The molecular underpinning for the hetero geneity in clinical prognosis of different tumour subtypes remains largely unclear and will be a major focus of future research. Distinct metastatic potentials observed in these cancer subtypes may simply reflect the cumulative effect of a diverse range of initiating and contributing oncogenic mutations. In a different, but not mutually exclusive, scenario, the same oncogenic insults may take place in different cell lineages, such as in basal versus luminal epithelial cells[40–42], or during different stages of differentiation, such as adult tissue stem cells versus differentiated progenitor cells, and this may ultimately influence the malignant and metastatic potential of the resulting tumours. Indeed, the introduction of identical genetic elements into two independent normal human mammary epithelial cells led to tumour xenografts with distinct lung metastatic properties[43]. Such findings strongly suggest that the pre-existing cellular programmes among distinct epithelial cell types can influence the ultimate cancer phenotype irrespectively of subsequent genetic changes. Accordingly, exploring the role of adult tissue

stem cells and stem cell-like tumour-initiating cells in the development and maintenance of metastatic capabilities is becoming an active area of investigation[7,44]. To date, transgenic model systems that introduce specific genetic elements into individual cell types of a particular tissue have been used to identify the cell of origin for several cancers[45–47]. For example, the inactivation of *Rb1* and *Trp53* in distinct pulmonary cell types recently showed that neuroendocrine and, to a lesser degree, alveolar type 2 cells are responsible for the development of small-cell lung cancer[48]. The picture is less clear in basal cell carcinoma, as two separate transgenic models revealed different cells of origin in the skin epidermis[49,50]. These seemingly incongruent results may be reconciled if each model were shown to represent a distinct clinical subtype of basal cell carcinoma. Future research harnessing the power of sophisticated animal models of tumour initiation and progression will be integral to studying the relationship between the cell of origin and the metastatic abilities of different cancer subtypes. As we explore the diversity of cell lineages and cellular hierarchy during organ development, tissue homeo stasis and oncogenesis, we will better understand the cellular contexts that allow the acquisition of metastatic functions.

Beyond the genetic and cellular heterogeneity of tumour cells, the inherent diversity in the host genetic background has also been recognized to influence metastatic risk. A pioneering study showed that an identical oncogenic event in the mammary tissue of mice with different genetic backgrounds led to the development of mammary tumours with similar primary tumour properties but with distinct lung metastasis proclivities[51]. Linkage analysis further mapped several genomic loci that modify metastatic potential in mice[52–54]. Future studies may extend this area of research to human patients with the application of genome-wide association study (GWAS) methods. By understanding the relative influence of a patient's genetic background on their risk of developing metastatic disease, we will be better prepared to implement appropriate therapeutic options early on in treatment.

Identifying drivers of metastasis.

As prognostic gene signatures were being discovered, gene expression profiling was concomitantly being applied to identify functional drivers of metastasis (TABLE 1). This area of research was built on decades of groundbreaking work by Isaiah Filder, Fred Miller and many others who focused on deriving organ-specific metastatic sublines of tumour cells through *in vivo* selection or clonal expansion. Comparing the expression profiles of highly metastatic cells with their weakly metastatic counterparts from an isogenic background allowed for the efficient and unbiased identification of candidate regulators of metastasis, including metastasis-promoting[18–20,55–57] and metastasis-suppressing genes[58–60]. Furthermore, gain-of-function or loss-of-function genomic screens, cross-species integrated genomic analyses and computational reanalysis of genomic profiling data have also led to the identification of functional mediators of metastasis with direct clinical relevance[61–64] (TABLE 1).

As highlighted above, advances in massively parallel sequencing technology have vastly improved our capacity to more comprehensively uncover genomic changes that underlie cancer development and progression[65,66]. Next-generation sequencing of matched primary tumours and metastasis or isogenic cell lines with different metastatic abilities, together

with the characterization of transcriptomic and epigenomic alterations in cancer through RNA sequencing and chromatin immunoprecipitation (ChIP)–sequencing, respectively, will help to reveal previously unidentified genomic disturbances that potentially contribute to malignant progression. The considerable challenge that remains is the functional interpretation of these genetic and epigenetic changes. Considering the magnitude of alterations found in cancer genomes, this task seems daunting; however, sophisticated computational algorithms that are currently being explored, such as those that use sequence-based and structure-based predictive features (such as PolyPhen2), should aid in distinguishing the driver mutations from the passenger mutations and will probably reduce candidate gene lists by appreciable amounts[67–69]. Relevant genomic alterations should be translated into gene expression and protein level phenotypes, which can be predicted using computational models, but which will ultimately rely on fundamental molecular and biochemical approaches. Following characterization, these molecular phenotypes can be placed into the context of subcellular networks and pathways with an emphasis on functional consequences (discussed below). Finally, the altered cellular phenotypes should be connected to metastasis-promoting features and validated using clinically relevant experimental model systems. Ultimately, harnessing the power of next-generation sequencing will certainly facilitate our understanding of metastatic evolution.

Proteomics.

Direct exploration of protein-level variations using modern proteomic techniques[27,28] has emerged as another powerful tool in the investigation of cancer metastasis. A key advantage of the proteomic approach is the ability to examine the biochemical, cellular and sometimes even organismal phenotypic states rather than genotypic expression patterns. Mass spectrometry can systematically analyse several thousands of proteins with quantitative precision through the combination of stable isotope labelling by amino acids in cell culture (SILAC)-based proteomic techniques and advanced bioinformatics. In recent years, quantitative proteomics has been applied to identify proteins that are differentially expressed in separate cellular compartments, such as those found in the membrane and/or secreted by the cell (the subset of the proteome known as the secretome) of highly metastatic versus weakly metastatic cells[70–74], although the functional importance of such proteins has not been thoroughly investigated. Furthermore, comparing the proteome of plasma, serum and/or urine from patients with metastatic cancer with those from patients with localized disease can aid in the recognition of prognostic biomarkers, a subset of which may be drivers of cancer progression and thus strong candidates for therapeutic intervention[75]. These approaches have not entered mainstream medicine owing to current shortcomings and inherent challenges such as the low sensitivity and specificity of existing cancer biomarkers[76]; a poor understanding of the biological and pathological importance of protein dynamics (changes in protein quantity and post-transcriptional modifications, for example); limited access to high-quality biospecimens; a lack of standardized methodology in discovery and validation studies; the inadequate incorporation of biomarker assessment in current clinical trial designs; and insufficient collaboration among proteomic, biological and clinical teams, as well as institutions.

Despite the multifaceted hurdles that riddle the path of proteomics en route to routine clinical use, the potential of proteomics to revolutionize metastasis research and patient care cannot be underestimated. At present, proteomic studies have yet to fulfil their potential of providing the functional insight that has been achieved by classical genomic studies, and therefore proteomic studies represent an area of investigation that warrants greater effort and resources in the coming years. Future studies will need to evaluate biomarkers for pre-cancerous lesions, localized disease and/or micrometastases in an effort to support early diagnosis and prognosis: these molecules may be very different from those that are found in advanced disease. Proteomic studies of microdissected clinical specimens should reveal intricate signalling networks that exist between the tumour and stromal compartment at a level that is unattainable by genomic profiling and sequencing studies alone. Ultimately, a multidisciplinary and systems approach will provide the most insightful and comprehensive understanding of cancer metastasis.

Towards systems biology of metastasis.

The application of genetic and molecular biology techniques to the investigation of cancer metastasis has yielded the vast majority of discoveries over the past few decades. As such, cancer metastasis has largely been explained through reductionism; that is, it has been defined by individual genetic disturbances and their resultant cellular phenotypes. Considering the complex nature of metastasis, a more holistic approach to its investigation, perhaps through systems biology, seems to be essential (FIG. 1).

Signalling pathways in cancer metastasis have been extensively studied at the level of individual proteins or as a linear cascade of proteins but they have been less frequently evaluated through a network approach. High-throughput data can be extracted from and annotated on the basis of comprehensive genomic, transcriptomic and proteomic interrogation of experimental or clinical samples[65,77]. The information we gather from these large-scale techniques can be used to develop network maps, interactomes, ensemble descriptions and gene expression modules with the assistance of advanced computational algorithms[78]. These network models can be validated, using a myriad of experimental methods, ranging from basic biochemistry looking at protein–protein interactions to sophisticated molecular real-time imaging examining signalling pathway activity in live tissue[79]. Importantly, the response of these networks to stimuli, which can now be measured through multiplex technologies such as multiparameter flow cytometry, and selective perturbations of individual components using small interfering RNA (siRNA) or drug compounds, is integral to establishing functional network models[28]. Ultimately, these multifunctional models can be used to discover novel signalling proteins, predict therapeutic response to selective inhibitors and uncover resistance mechanisms. For example, the drug–gene–phenotype Connectivity Map approach[80] was successfully used to identify the mTOR inhibitor rapamycin as an effective agent for overcoming dexamethasone resistance in acute lymphoblastic leukaemia.

Two active areas of investigation in cancer metastasis that could benefit greatly from a systems biology approach are micro-RNA (miRNA) and epigenetic regulation. Of note, these cellular programmes are not independent of each other, as DNA methylation has been

shown to silence miRNA activity[81–83] and miRNAs have been shown to affect epigenetic changes[84,85]. miRNAs have predominately been implicated in cancer metastasis through their regulation of the early steps in tumour progression, such as migration and invasion[86–88], but more recently through their effect on later stages, such as colonization[89]. miRNAs are commonly linked to their metastatic functions by regulating individual genes that are involved in metastasis. However, miRNAs, like transcription factors, function as master regulators that can simultaneously control the expression of several hundred genes and also affect dramatic shifts in cellular phenotype[90,91]. As such, mapping out individual genes that are targeted by miRNAs and other non-coding RNAs[92] may not faithfully represent the extent of their influence. Instead, the breadth of genes regulated by an miRNA can be collected using genomic and proteomic approaches and subsequently translated into ensemble data sets with the help of computational algorithms that effectively group specific genes into functional modules. These modules can further be used to generate network models that will optimally reflect a more comprehensive picture of the cellular and behavioural functions that are affected by an miRNA and can ultimately provide a better insight into how miRNAs may affect cancer metastasis. Once these networks are established, the discovery of future cancer-promoting genes and their regulation by miRNAs can be placed in context.

Epigenetics has been implicated in cancer progression through its regulation of tumour initiation, stem-cell properties and, rather intriguingly, chronic inflammation[85,93–96]. Similar to miRNAs, epigenetic regulation modulates a broad range of coordinated genome-wide expression changes, but does so at a different level from conventional genome aberrations that are associated with cancer progression. The direct contribution of epigenetics to cancer metastasis is fairly unexplored, but we are now starting to make headway with the support of high-throughput genomic studies. For example, genome-wide methylation analysis of paired colorectal cancer primary tumour and liver metastasis specimens demonstrated differences in DNA methylation status in advanced cancer at a global and individual gene level when compared with localized disease[97]. However, the relationship between metastasis-related epigenetic differences and corresponding changes in gene expression or cellular function has not been defined. Using computational algorithms[98], a systems level approach can help to incorporate the unique epigenetic-mediated gene expression and cellular changes in the context of previously defined genomic and proteomic alterations that are associated with metastasis. A systems level understanding of regulatory programmes that govern metastatic behaviour will ultimately need to be integrated within the context of tumour–stromal interactions that occur at different stages of tumour progression.

Cellular heterogeneity of metastasis

Understanding the contribution of stromal cells to cancer metastasis is essential to fulfil the promise of improved therapy, as foreseen by the seed and soil hypothesis. Here, we base our discussion on some of the key conceptual advances that have been made in the past decade regarding the role of stromal cells and cellular dynamics during different phases of metastasis. For more in-depth coverage of these topics see REFS 5,11,99.

Effect of the primary tumour microenvironment on metastasis.

Although the ability of non-neoplastic stromal cells to promote tumour proliferation, invasion, survival and chemoresistance is well known[14,15,100–106], we are only now beginning to recognize their function in the development of cancer metastasis. It should not come as a surprise that tumour angiogenesis was among the initial findings that supported a role for stromal cells in cancer metastasis; the poor vascular integrity of newly synthesized blood vessels within the tumour allows for the escape of malignant cells with the potential of distant spread. As mediators of tumour angiogenesis were uncovered, targeted therapies against these molecules were designed and among the first to achieve clinical application for the control of late-stage metastatic disease[107–109]. Although anti-vascular endothelial growth factor (VEGF) therapy was approved to combat metastatic disease in patients[110], recent studies in mice paradoxically revealed an increased risk of metastasis associated with this therapy[111,112], which underscores the peril of oversimplifying the potential effect of targeting the tumour stroma. These preclinical findings corroborate results from recent clinical trials showing no overall survival benefit for the VEGF inhibitor bevacuzimab in various cancers[113–115], bringing its therapeutic value into question[116] and serving as a sobering reminder to consider the unexpected consequence of anticancer therapy, particularly in regards to metastasis.

In more recent years, we have witnessed important roles for additional primary tumour stromal cell types in cancer metastasis. Elegant studies combining intravital imaging and mouse modelling have convincingly demonstrated a pro-metastatic role of tumour-associated macrophages through colony stimulating factors (CSFs) and epidermal growth factor (EGF) signalling[117,118]. More recent reports have implicated additional pathological and molecular mechanisms that mediate crosstalk between tumour cells and macro phages, ultimately influencing cancer metastasis[119,120]. Leukocytes and other immune cells have been recognized as crucial regulators of primary tumour growth and metastasis[121–123]. Mesenchymal cells that reside in breast tissue have also been shown to affect the metastatic behaviour of breast cancer cells through CCL5 signalling[13]. Moreover, a recent report showed that mesenchymal cells can influence the metastatic behaviour of neuroendocrine small-cell lung cancer. Most intriguingly, the mesenchymal cells and neuroendocrine cells were descendants of the same progenitor cell, which exploited RAS signalling to generate functional intra-tumoural heterogeneity[124]. These studies have established stromal cells as important regulators of metastasis through their ability to influence cancer cell functions such as chemotaxis and invasion, as well as microenvironment properties, such as vessel integrity and the presence of immunological cells. Despite these advances, we have yet to discover many of the molecular components that facilitate communication between tumour cells and individual stromal cells of the primary tumour. We need a better working knowledge of the paracrine signalling network that mediates these molecular interactions — an area of research that will be facilitated by advanced proteomics — as these interactions are key components in our systems level understanding of metastasis. There is also limited insight into how multiple stromal components concomitantly associate with tumour cells. For example, what is the significance of inter-stromal crosstalk between different lineages of stromal cells in malignant progression? How does stromal heterogeneity synergize with tumour heterogeneity to encourage metastatic spread? Importantly, advances in molecular

imaging and microscopy have opened new avenues of investigation for better examining the contributions of cellular heterogeneity in cancer progression (BOX 2).

Tumour cells in transit.

Essential to cancer metastasis is the ability of primary tumour cells to enter the vasculature and to use these fluid ‘highways’ as a means to reach distant organs. Seminal studies by James Ewing highlighted the influence of the vascular anatomy on the ultimate destination for metastatic tumour cells[3]. Although we now understand that the vasculature alone does not explain the pattern and distribution of metastasis, the ability of tumour cells to endure substantial stress in transit remains a poorly understood aspect of metastasis. Even more, we have yet to characterize how this selection pressure affects the subsequent metastatic behaviour of surviving cells. Platelets have been recognized as an important blood component that protects circulating tumour cells (CTCs) and promotes their metastatic colonization[125]. Nevertheless, knowledge regarding the cellular and molecular mechanisms that allow tumour cells to survive and adapt within circulation remains scarce, owing in part to the inherent difficulty in isolating and analysing CTCs. Recent technical and conceptual advances have helped overcome these limitations.

The most effective strategies in isolating tumour cells from the circulation have relied on antibody-based epitope capture methods[126]. The application of microfluidic rare cell detection approaches has improved CTC purity and yield in recent years[127]. Although the technical challenges of detecting CTCs remain, rapid advances in bioengineering are rendering these hurdles surmountable. We already appreciate the clinical value of CTCs, as recent studies have associated their presence in the bone marrow with poor prognosis in patients with breast cancer[128,129]. On the basis of these findings, the detection of CTCs has been incorporated into the international tumour staging systems[130] and endorsed through recent recommendations on tumour markers made by the American Society on Clinical Oncology (ASCO)[131].

Despite their clear prognostic importance, the diagnostic value of CTCs is largely unknown and fairly unexplored. We have not defined whether CTCs can be reliably detected before the development of metastatic disease. Our ability to diagnose and to manage prostate cancer would clearly benefit from this understanding: the surveillance and molecular analysis of CTCs at regular intervals in patients with localized prostate cancer could help to distinguish indolent disease from aggressive disease and could ideally inform subsequent treatment decisions. Moreover, the ability of CTCs to predict clinical response to therapy would also help to guide disease management, as shown by examples in breast cancer and non-small-cell lung cancer[127,132]. As such, many of the advantages and strategies of clinical proteomic and next-generation sequencing discussed above can be adapted to the evaluation of CTCs.

There are also many unresolved questions concerning the biology of cancer metastasis that may reveal their answers in the exploration of CTCs. For example, how does the genomic and proteomic landscape of CTCs compare with their corresponding primary tumour or metastasis? Are there unique gene expression changes in CTCs that are not found in the primary tumour or metastasis? Do these expression patterns implicate independent signalling pathways or cellular phenotypes? Are these expression changes associated with stem-cell

properties, epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET), or other metastasis-promoting functions? Can these genomic or expression patterns be exploited for drug development? The answers to these questions will improve our understanding of metastatic evolution and will resolve different schools of thought with respect to cancer progression, such as the linear versus parallel progression models (BOX 1).

Tumour–stromal interactions in distant organs.

The colonization and outgrowth of tumour cells in a secondary organ is often considered the rate-limiting, as well as the most poorly delineated, step in the metastatic cascade. We have begun to elucidate the basis of metastatic colonization by characterizing the functional involvement of the tumour stromal cells of the secondary site[5]. Considering the vast area of research that is encompassed by this subtopic, we focus on the salient advances that may help to direct future research.

The emerging concepts of the premetastatic niche[99,133] and metastatic selfseeding[134,135] have challenged our traditional view of metastasis and have stimulated new avenues of research (FIG. 2). Metastasis is no longer considered a unidirectional flow between primary tumours and distant organs. The pre-metastatic niche model shows that, preceding the arrival of disseminated tumour cells (DTCs), bone marrow-derived haematopoietic stem cells are mobilized by tumour-derived factors and are recruited to the secondary site where they negotiate a more hospitable microenvironment to foster the survival and expansion of metastatic lesions[133]. According to the self-seeding hypothesis, metastatic tumour cells can also return to the primary site, accelerating the growth and malignant evolution of primary tumours (FIG. 2). Further investigation using experimental and clinical models will help to define the precise role and mechanism of these events in metastasis.

Inflammatory cytokines have emerged as crucial mediators of the pre-metastatic niche and self-seeding[133,135–137]. The interplay between colonizing tumour cells and the microenvironment of the secondary organ also seems to involve inflammatory cytokines, exposing these molecules as prime targets for therapeutic intervention. Recent studies have shown that the tumour-induced secretion of interleukin-6 (IL-6) by stromal cells that reside in the bone and brain facilitates metastatic colonization[138–141]. Interestingly, inflammatory signals frequently affect tumour cells by altering their epigenetic regulatory programme and by conferring cancer-promoting properties[142], which highlights a previously identified area of research that could benefit from a systems biology approach. For example, an epigenetic switch that is initiated by the oncoprotein SRC was sustained by an inflammatory regulatory network involving IL-6 and nuclear factor-κB (NF-κB) signalling in tumour progression[85]. Intriguingly, an independent report showed that SRC signalling was preferentially activated in latent breast cancer cells and mediated pro-survival responses to inflammatory cytokines that supported bone metastasis. Associating these findings within the context of many others, we recognize that inflammation-induced genetic and epigenetic changes may contribute to the survival of disseminated cancer cells and may represent a principal topic of future metastasis research and therapeutic development.

After surviving the adjustment to the secondary site, tumour cells must sustain their growth to develop overt metastases. Developmental pathways have emerged as

important players in tumour progression and metastasis[143]. Although transforming growth factor-β (TGFβ)[18,144,145], bone morphogenetic protein (BMP)[146–151], WNT[152–157] and Hedgehog[158–160] signalling have all been shown to influence bone metastasis, the Notch pathway has recently joined the ranks through a stroma-dependent mechanism that was susceptible to pharmacological inhibition[140]. In lung cancer, cell-autonomous activation of the WNT–TCF transcriptional programme was shown to promote brain and bone metastasis through the actions of LEF1 and HOXB9 (REF. 161). Furthermore, both the Notch and WNT pathways were recently shown to participate in generating a viable metastatic niche for lung-colonizing breast cancer cells through the actions of the extracellular matrix protein tenascin C[162]. It is important to note that, similar to their pleiotropic function during cell fate decisions in metazoan organisms, developmental signalling pathways often regulate multiple metastasis genes with diverse functions that facilitate organ-specific metastasis, and therefore developmental signalling pathways represent key regulatory nodes in the metastasis network. A case in point is the TGFβ pathway, which activates the expression of prometastasis genes *VEGF*, angiopoietin-like 4 (*ANGPTL4*), *JAGGED1*, matrix metalloproteinase 1 (*MMP1*), *CXCR4*, parathyroid hormone-like hormone (*PTHRP*), *IL6* and connective tissue growth factor (*CTGF*). The perspective of metastasis as a developmental programme that has gone awry will continue to be a major topic of research with important therapeutic implications.

Clinical translation

As most metastatic cancers are inoperable, systemic treatments using chemotherapeutic or targeted therapy is often the only option to slow tumour growth or to relieve metastasis-associated morbidity. Although agents that target tumour-specific pathways have been, and will continue to be, major components of anti-metastasis therapy, treatment strategies targeting the tumour microenvironment of the secondary organs will greatly augment our ability to treat late-stage cancer.

Tumour cell-targeted therapy.

The ultimate goal in the treatment of metastatic cancer is to achieve sustained disease remission, which in theory would require the eradication of all cancer cells, regardless of their systemic distribution. With this in mind, genes and pathways that have crucial roles in primary tumour growth and metastasis are ideal targets for therapeutic inventions, as they are likely to show efficacy in reducing both tumour burden and metastasis risk. For example, rigorous research has shown that oncogenic BRAF signalling has an important role in the pathogenesis of malignant melanoma[163–167], and this prompted clinical trials for specific and potent inhibitors of mutant BRAF, the initial results of which suggest dramatic efficacy in the treatment of metastatic malignant melanoma[168,169]. Despite substantial successes in targeted cancer therapy, the chronic problem that will continue to plague the future of targeted therapeutics is drug resistance. The mechanisms underlying resistance to targeted therapy are under active investigation[170–178]. Overcoming drug resistance will depend on these characterizations, as well as on using the knowledge that we have gained from previously defined effective strategies, such as second-generation ABL inhibitors[179–181]. Similar to the story of first-generation ABL kinase inhibitors in chronic myeloid leukaemia,

the effects of BRAF inhibitors, although profound, are only temporary owing to resistance. Two recent studies[182,183] elucidate the potential mechanisms that underlie resistance to BRAF inhibitors and thus provide evidence encouraging novel avenues for rational drug design of second-generation BRAF inhibitors. As new therapies for cancer metastasis are discovered, the field investigating drug resistance will grow dramatically.

Targeting the tumour microenvironment.

As discussed above, the cellular microenvironment of the secondary site has a crucial role in facilitating cancer metastasis. Often, the influence of the microenvironment, and the cellular and molecular adaptations undertaken by tumour cells to successfully colonize a secondary organ, alter metastatic cells in ways that render them resistant to cell-autonomous therapies that effectively treat their corresponding primary tumour[184,185]. Moreover, even when well-accepted cell-autonomous mechanisms of drug resistance are defined, as in the case of BCR-ABL, alternative methods of drug resistance that are driven by the microenvironment are still largely at work[186]. Experimental mouse models have shown the therapeutic inadequacies of targeted agents in treating metastatic lesions[187,188]. These observations and findings collectively support the rationale for targeting the microenvironment of the metastatic lesion in conjunction with targeting the tumour cells directly to better treat metastatic disease.

Our progress in treating bone metastasis by targeting molecules that are found in the tumour microenvironment, such as RANKL[189,190], is a direct consequence of the insight gained from systematically dissecting the intricate molecular and cellular crosstalk between tumour cells and bone stromal cells. Elucidating the homeostatic balance between osteoclasts and osteoblasts in the bone microenvironment has been paramount in establishing a contextual basis for understanding bone metastasis and developing targeted therapies[17]. In addition, the characterization of the haematopoietic niche[191] and its interactions with bone cells is likely to help us to understand the potential existence of metastasis niches that have been speculated to facilitate the survival and expansion of DTCs in bone. Thus, a sound framework of normal homeostatic mechanisms can improve our ability to understand and target tumour–stromal interactions in metastasis.

Future directions

We have made significant progress over the past decade in harnessing new technology and research tools to piece together an intellectual framework for understanding cancer metastasis. Moving forwards, efforts should be focused on closing some of the major gaps in areas of metastasis research that have important implications for therapeutic development. For example, the best window of opportunity to control metastatic disease may be the time period between metastatic seeding and the clinical detection of overt metastasis, as this time period represents an occasion when tumour cells are likely to be vulnerable to therapeutic agents and patients are expected to be in an optimal physical condition to endure treatment. However, despite our increasing knowledge about metastatic colonization, we still hold little understanding of how metastatic tumour cells behave as solitary disseminated entities, particularly at crucial junctures in their dynamic existence, such as during the establishment

of micrometastases, activation from latency and response to therapeutic regimens. We will need to resolve tumour–stromal dynamics at the single cell level using advanced imaging technology, so that the fate of individual tumour cells and the different lineages of stromal cells, as well as their molecular interactions, can be traced during disease progression and drug treatment. The understanding that we gain from these studies could facilitate the generation of clinically relevant cancer models with the potential to guide the direction of drug development.

A systems biology approach for identifying linker components or key regulatory nodes that connect different functional networks and processes may help us to integrate the components of tumour progression with the individual steps of the metastatic cascade, leading to a more fluid, interdependent depiction of metastasis (FIG. 1). Network modelling can be correlative and therefore considered a descriptive science that often requires further investigation to establish causal relationships. For systems biology to thrive, there will need to be a shift in culture, especially from the funding and peer-reviewed perspective, to encourage a more global understanding of disease.

Systems level investigations will undoubtedly reveal many possible directions for therapeutic exploration. A potential bottleneck is the requirement to functionally validate individual candidate genes cost-effectively and efficaciously in clinically relevant animal models[192–196] (TABLE 2). Xenograft models, which have played a predominant part in metastasis research, need to be supplemented with robust transgenic and knockout mouse models. Furthermore, humanized mouse models in which components of mouse stroma are replaced with human counterparts are proving to be valuable tools. By integrating the insight gained from these distinct animal models, we will improve our ability to characterize candidate metastasis genes. In particular, we will be able to understand their normal physiological roles, define their precise functions during different stages of tumour progression and evaluate their effect on therapeutic targeting. Extensive preclinical validation of candidate metastasis genes using robust animal models will increase the success rate of developing effective anti-metastasis agents while minimizing the risk of unexpected adverse side effects.

We also need to renovate the design of clinical trials to expedite the development and approval of anti-metastasis therapies. Owing to the considerable financial burden of testing therapeutic agents, clinical trials are seldom designed to evaluate anti-metastasis therapy in the setting of early stage cancer, which may represent a point in disease progression when metastasis might be preventable. As such, many effective anti-metastasis agents that are already US Food and Drug Administration (FDA)-approved for other indications in the United States have not benefited cancer patients who are at risk for metastasis. Reliable and specific biomarkers that reflect an accurate readout of disease progression and efficacy of therapeutic targeting should be implemented in clinical trial design. This will improve our ability to measure the effectiveness of drug targeting, select the optimal patient population for therapeutic intervention and gain accelerated regulatory approval. Finally, clinical trial design should incorporate standardized procedures for the collection of patient tumour samples from localized and metastatic disease at distinct clinical stages, as well as during different points in disease management (such as pre-therapy and post-therapy). These

invaluable specimens, including their associated molecular profiles, should be deposited in publically available tumour and data banks to facilitate multidisciplinary research and collaborations. With concerted effort from basic researchers, clinical investigators, drug developers and regulatory agencies, considerable improvements in the management of metastatic cancer may be within reach. We foresee a future in which patients at a high risk of metastasis will be reliably identified using molecular profiles of their primary tumour and CTCs. Effective cocktails of drugs with minimal adverse side effects will be tailored to prevent metastatic recurrence in individual patients. Even for patients with advanced cancer, the diagnosis of metastasis will no longer carry the label of a terminal illness, but will rather be acknowledged as another complex chronic condition that can be effectively controlled with a large arsenal of effective therapeutic agents.

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Box 1 |

Linear and parallel progression models

According to the linear progression model, primary tumour cells undergo successive rounds of mutation and selection[35], giving rise to a biologically heterogeneous cellular population in which a subset of malignant clones have accumulated genetic alterations, necessary for metastasis[197,198]. The metastatic capabilities may be developed at the primary site as a by-product of the selective pressures, or may further evolve after the tumour cells reach the secondary organs. Pioneering work by Isaiah Fidler in the 1970s demonstrated that only a subset of pre-existing cells in a heterogeneous primary tumour can successfully metastasize[199]. From a clinical perspective, a direct correlation between tumour size and frequency of metastatic events[200,201], in addition to the reduction of metastatic risk by the surgical resection of tumours that are <2cm in size, also support the linear progression model.

By contrast, the parallel progression model argues that tumour cells may disseminate very early in malignant progression, colonize multiple secondary sites at different times and ultimately accumulate genetic changes independently from those incurred by the primary tumour[8]. Select studies comparing the growth rates of primary tumours and their secondary lesions concluded that metastases were too large to be initiated during advanced stages of cancer progression[202,203]. A more recent report provides evidence supporting the early dissemination of metastatic tumour cells in transgenic mouse models of breast cancer[204]; however, the competence of these disseminated tumour cells in forming secondary lesions is currently under investigation[30]. As such, these two competing, but not mutually exclusive, paradigms provide a conceptual basis for the investigation of metastatic evolution, and have important implications in the prevention and treatment of metastatic disease[8].

Box 2 |

Imaging cancer dynamics

Currently, the mainstay objective of imaging modalities in the clinical setting is to characterize the extent of metastatic disease in cancer patients, which has been greatly refined by the integration of diverse macroscopic imaging modalities. Positron emission tomography (PET) has been combined with computed tomography (CT) imaging to improve our ability to stage metastatic disease and to monitor response to treatment[205,206]. The simultaneous use of multiple isotope-labelled probes, such as the combination of ^{18}fluoride and ^{18}fluorodeoxy glucose (^{18}FDG), in conjunction with hybrid imaging modalities, has further enhanced our ability to detect metastatic disease[207]. Moreover, cancer-specific and organ-specific molecular probes, such as those used in the detection of melanoma using PET and liver metastasis using magnetic resonance imaging (MRI), respectively, hold great promise in augmenting the resolution with which we can detect systemic cancer spread[208,209].

At the bench, we have come a long way from direct visualization of tumour cell invasion using green fluorescent protein[210]. For example, direct visual evidence of specific cell lineages, such as cancer stem cells, in metastasis can be obtained using high-resolution, non-invasive imaging[211]. The combination of a mammary imaging window and a photo-switchable fluorescent protein has been used to investigate the influence of spatially and functionally distinct tumour microenvironments on cancer cell invasion and intravasation[212,213]. The simultaneous observation of distinct stromal cells as they interact in the primary tumour can be achieved through multiphoton microscopy, which has the benefits of enhanced tissue depth and multiple colour channels[21,23,214].

Probing beyond the cellular level, there is now evidence that molecular imaging will provide functional readouts of subcellular biological processes, such as protein–protein interactions[215]. These modalities can be used to visualize real-time *in vivo* subcellular events that regulate tumour metastasis. For example, non-invasive imaging techniques together with tumour cells genetically engineered to provide functional readouts of a signalling pathway can be used to test targeted anti-metastatic agents in preclinical metastasis models[216]. Importantly, these powerful tools have been integrated with computational programs to provide quantitative readouts in addition to high-resolution, real-time qualitative impressions. In the future, the sophistication of molecular imaging may also translate into the clinical setting to provide instant readout and direct visualization of biological processes that promote tumour metastasis[217].

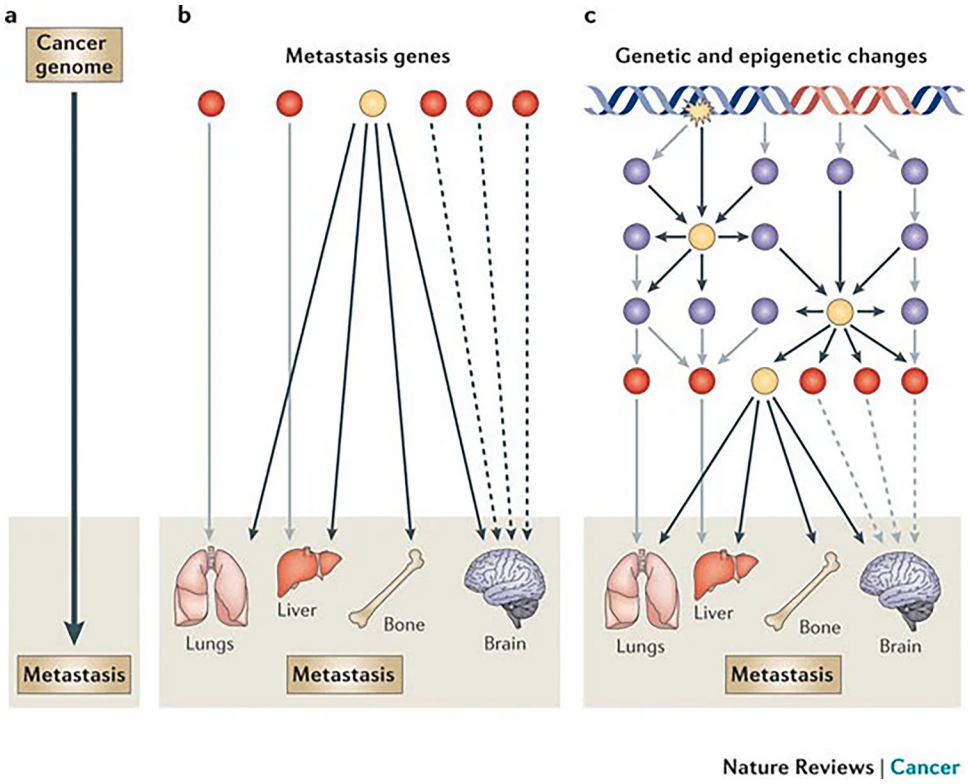


Figure 1 |. Research strategies for understanding the molecular basis of cancer metastasis: from reductionism to systems biology.

The evolving states of cancer, including metastasis, are reflected in dynamically changing expression patterns of the genes and proteins within the cancer cells. Metastasis research has generally relied on the linear approach of gene-to-trait mapping, which links the metastasis genotype with its corresponding metastatic phenotype (part **a**). More complex linear linkages may include multivalent relationships, in which one gene (or group of genes) can have functions in multiple metastasis phenotypes (pleiotropy), and a metastasis tissue tropism can be exerted by many independent genes or gene groups (redundancy) (part **b**). However, gene interactions are influenced by their context, which often cannot be captured by traditional one-gene-one-trait approaches. Therefore, metastatic behaviour should be considered as the consequence of the collective action of individual metastasis genes through nonlinear interactions (part **c**). Understanding the nature of these network level interactions and identifying crucial nodes of functional control will pave the way towards rational therapeutic design for metastatic breast cancer. Red circles represent genes or groups of genes that mediate tissue-specific metastasis of breast cancer. Blue circles represent regulators of metastasis genes. Yellow circles represent key functional nodes of metastasis regulation networks and are prime targets for therapeutic development. The black, grey and dashed arrows indicate different pathways.

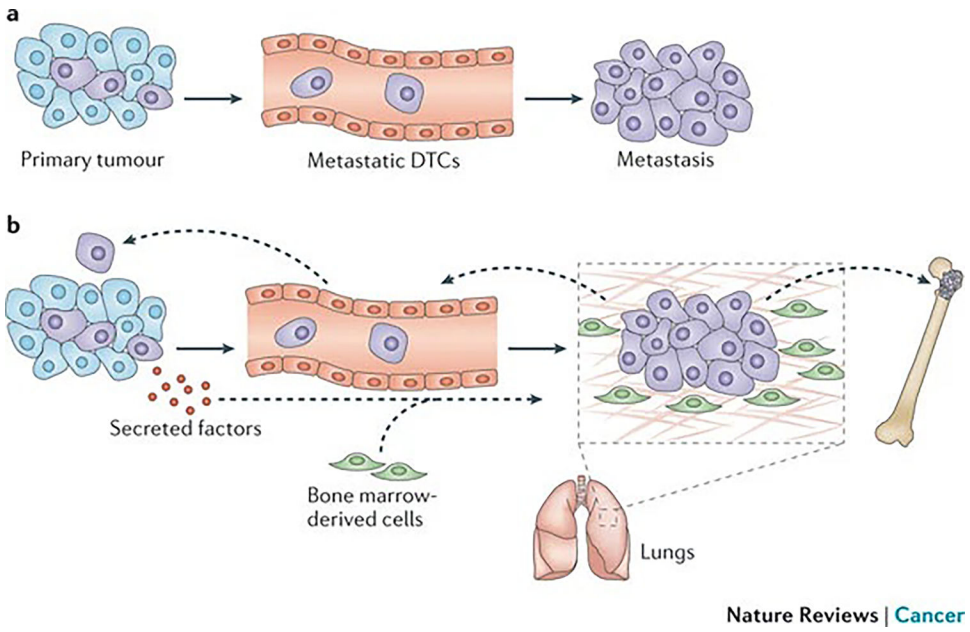
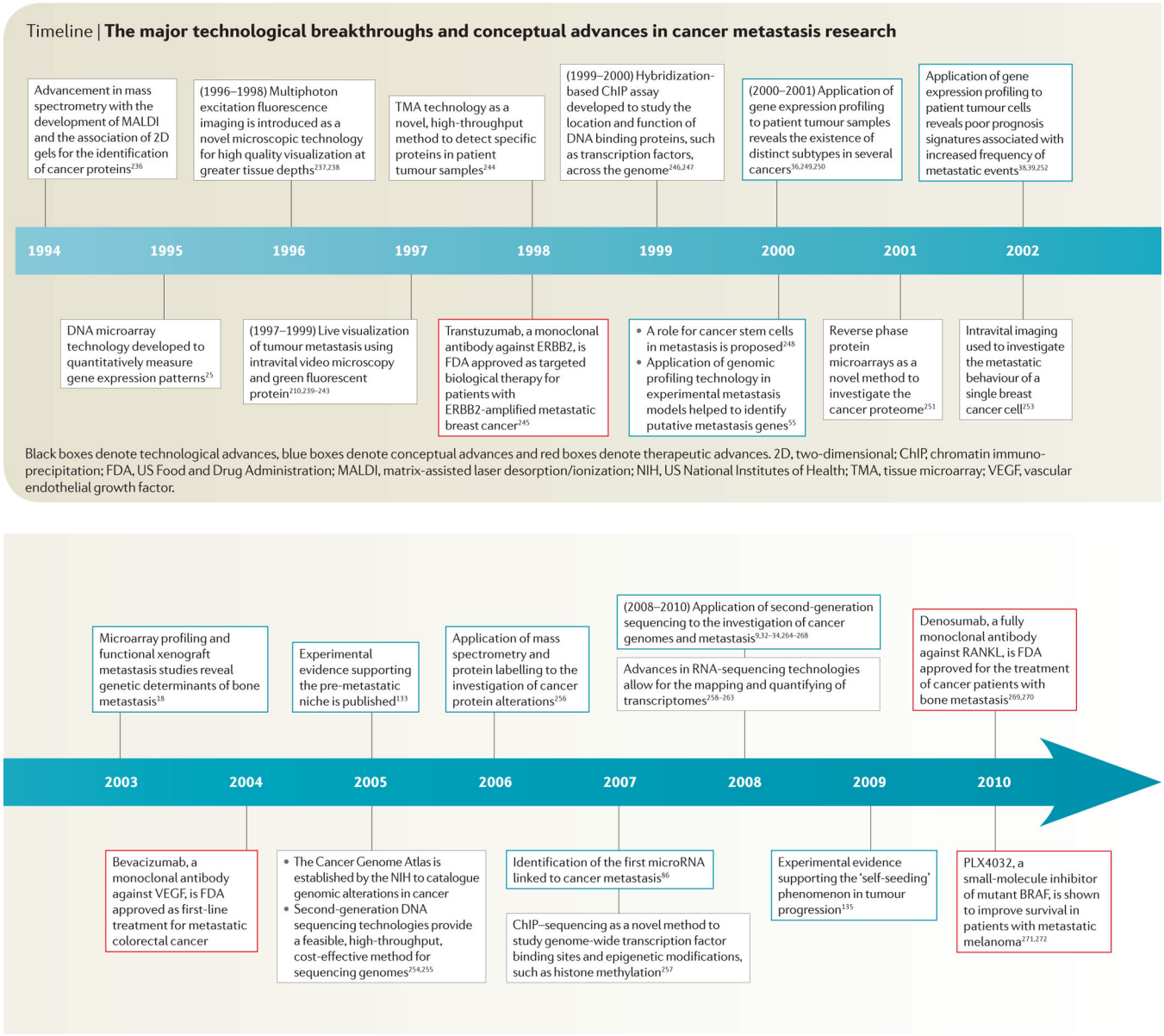


Figure 2 |. Evolving view of the dynamic relationship between the primary tumour and metastasis.

a | The traditional view of cancer metastasis in which primary tumour cells escape their site of origin, travel in a unidirectional path away from the primary site and ultimately colonize distant organs to give rise to systemic disease is shown. **b** | A dynamic view of cancer metastasis in which bone marrow-derived cells are mobilized by tumour-derived inflammatory factors and prime distant sites of metastasis to form the pre-metastatic niche is shown. Disseminated tumour cells (DTCs) (either from the primary tumour or from metastases) that have been selected with enhanced malignancy can colonize distant organs, as well as repopulate the primary site through the phenomenon of self-seeding.



Timeline |. The major technological breakthroughs and conceptual advances in cancer metastasis research

Black boxes denote technological advances, blue boxes denote conceptual advances and red boxes denote therapeutic advances. 2D, two-dimensional; ChIP, chromatin immunoprecipitation; FDA, US Food and Drug Administration; MALDI, matrix-assisted laser desorption/ionization; NIH, US National Institutes of Health; TMA, tissue microarray; VEGF, vascular endothelial growth factor.

Table 1 |

Select general and organ-specific metastasis genes uncovered by novel approaches

Genes	Function	Approach	Refs
Bone metastasis			
IL11 and *CTGF*	TGFβ target genes that functionally promote metastasis tothe bone	Genomic profiling of bone-metastatic variants after *in vivo* selection in experimental mouse model	18
ADAMTS1 and *MMP1*	Metalloproteinases that proteolytically release EGF-like ligands from tumour cells that downregulate OPG levels in osteoblasts through EGFR signalling	Genomic profiling of bone-metastatic variants after *in vivo* selection in experimental mouse model	220
JAGGED1	Increases IL-6 production from osteoblasts to promote tumour growth, and stimulates osteoclast differentiation and activity	Expression profiling of metastatic variant cell lines and prognostic analysis of human clinical data	142
SRC	Promotes survival of DTCs in the bone microenvironment by inducing AKT-dependent survival cues to the inflammatory cytokines CXCL12 and TRAIL	Signalling pathway signature analysis of clinical profiling data	221
NF-κB [*]	Transcription factor that facilitates breast cancer bone metastasis through the osteoclast-promoting activity of GM-CSF	Surveillance of signalling pathway activity in bone metastatic cells	222
Lung metastasis			
EREG, COX2, MMP1 and *MMP2*	Act collectively to promote angiogenesis, intravasation and extravasation	Genomic profiling analysis of lung-tropic variants	223
ANGPTL4	Promotes extravasation by disrupting endothelial integrity	Genomic profiling analysis of lung-tropic variants and analysis of signalling pathway activity in clinical lung metastasis	224
ERP5	Promotes migration and metastasis that is dependent on ERBB2 and PI3K signalling	Forward genetic screen using an orthotopic mouse model of breast cancer	58
MTDH	Promotes chemoresistance and adhesion to the lung endothelium to facilitate metastasis	Phage display screening of lung-homing peptides and mathematical analysis of clinical genomic data set	62,225
ID1	Promotes tumour-initiating function in lung metastasis	Genomic profiling of lung-tropic variants from in vivo selection	20,226
TNC	Promotes lung colonization by engaging the Notch and WNT developmental signalling pathways in the formation of a metastasis niche	Gene expression profiling of lung-tropic variants and immunostaining of lung metastases from patients with breast cancer	164
KLF17	Inhibits lung metastasis by suppressing EMT	RNAi screen in a mouse model of lung metastasis	227
VEGFR1 [‡]	BMD haematopoietic progenitor cells expressing VEGFR1 establish the pre-metastatic niche in lung metastasis	Molecular profiling of stromal cells in pre-metastatic niche	134
S100A8 [*‡] and *S100A9* [‡]	Inflammatory proteins expressed by the lung which recruit myeloid cells to establish the premetastatic niche	Gene expression profiling of lungs primed by primary tumours	138,139
CCL5 [‡]	BMD mesenchymal progenitor cells secrete CCL5, stimulating primary breast cancer cells to metastasize to the lung	Cytokine profiling of the co-culture of BMD mesenchymal progenitors and breast cancer cells	13
MMP9 [‡]	Expressed by lung endothelial cells and macrophages on induction by primary tumours expressing VEGFR1 tyrosine kinase	Genetic knockout and transgenic mouse modelling combined with in vitro tissue analysis	228
CXCL12 [‡] and *CCL21* [‡]	Ligands found in the lung that bind to their respective chemokine receptors expressed by breast cancer cells	Gene expression surveillance of breast cancer cells combined with functional in vivo studies	229

Genes	Function	Approach	Refs
Brain metastasis			
ST6GALNAC5, COX2 and *HB-EGF*	Mediators of breast cancer metastasis that promote extravasation across the BBB and colonization of the brain	Genomic profiling of brain-tropic sublines derived from in vivo selection	19
MIF, IL8 and *PAI1*	Tumour-derived factors that activate astrocytes in the brain and induce expression of inflammatory mediators	Cytokine profiling of tumour and astrocyte cultures	141
STAT3	Promotes melanoma invasion, angiogenesis and metastasis to the brain	Signalling pathway analysis of in vivo selected bone metastatic variants	230
IL6, IL1B and *TNF*	Astrocyte-derived cytokines associated with the development of brain metastasis	Cytokine profiling of tumour and astrocyte cultures	141
General metastasis			
LEF1 and *HOXB9*	WNT and TCF target genes that promote metastasis to bone and brain through chemotactic invasion	Clinical data set analysis of signalling pathway activities	163
CXCR4 and *CCR7*	Chemokine receptors expressed on breast cancer cells that promote chemotaxis and metastasis	Gene expression surveillance of breast cancer cells with different metastatic abilities	229
RHOC	Promotes tumour invasion and metastasis	Expression profiling of isogenic melanoma cell lines with different metastatic abilities	56
TWIST	EMT-promoting transcription factor	Expression profiling of mammary tumour cell lines with different metastatic abilities	57
MACC1	Promotes proliferation, invasion and metastasis of colon cancer through HGF	Genome-wide expression analysis of primary and metastatic colon cancer	231
NEDD9	Promotes melanoma migration, invasion and metastasis by interacting with focal adhesion kinase	Genomic copynumber analysis in inducible transgenic mice	63
LKB1	Inactivation promotes lung cancer metastasis when combined with oncogenic KRAS, possibly by activation of SRC, P13K, and MEK1 and MEK2	Transgenic mouse models and clinical analysis of lung cancer; integrative genomic and proteomic studies using in vivo lung cancer models	64,65
LOXL1	Cell-autonomous role in promoting breast cancer invasion; systemic expression primes pre-metastatic niche for lung, liver and brain metastasis by recruiting CD11B+ myeloid cells	Gene expression profiling of hypoxia-induced genes and functional in vivo studies	232,233

BBB, blood-brain barrier; BMD, bone marrow-derived; *COX2*, cyclooxygenase 2; DTC, disseminated tumour cell; EGF, epidermal growth factor; EGFR, EGF receptor; EMT, epithelial to mesenchymal transition; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; *KLF17*, Kruppel-like factor 17; *MACC1*, metastasis associated in colon cancer 1; *MMP1*, matrix metalloproteinase 1; *MTGH*, metadherin; RNAi, RNA interference; *STAT3*, signal transducer and activator of transcription 3; TGFβ, transforming growth factor-β; *TNF*, tumour necrosis factor; *TNC*, tenascin C; *VEGFR*, vascular endothelial growth factor receptor.

*Nuclear factor-κB (NF-κB) is a transcription factor that can be formed by a number of different proteins.

≠Indicates stromal genes involved in metastasis.

Table 2 |

Mouse model systems used to investigate metastasis

Mouse model	Advantages	Disadvantages
Xenograft models	• Can accurately model tumour cell dissemination and metastatic colonization using experimental metastasis or spontaneous metastasis models • Human tumour cells derived from patients can be used • Multiple genetic manipulations can be used • They are amenable to *in vivo* real-time imaging with reporter genes	• Mice lack an intact immune system • Late-stage cancer cells are often required • There is some species-specific incompatibility • Established cancer cell lines that may evolve with passage *in vitro* are often used
Isograft or allograft models	• Can accurately model tumour dissemination and metastatic colonization • Mice have an intact immune system • Multiple genetic manipulations can be investigated • The models are amenable to *in vivo* real-time imaging with reporter genes	• Only mouse-derived cancer cells can be used • The resection of the primary tumour is often required before the development of metastases • It is difficult to randomize groups owing to variable primary tumour size and presence of local invasion • There is limited organ tropism
Germline traditional transgenic models	• Provide an accurate model of initiation, local invasion and the early steps of metastasis • Can be used for drug testing and development • Mice have an intact immune system • Can be used to analyse the potential effect of drug targeting on normal physiology	• Studies are often limited to lymph node and lung metastasis; these models do not develop bone metastases • There is a long lead-time in primary tumour and metastasis development • The development of metastatic lesions is asynchronous, making them difficult to track • There is incomplete penetrance of genetic modification • A tremendous amount of time, labour and resources are needed to generate mice with multiple genetic manipulations • A genetic mutation is usually present in all tissues, making it difficult to tease out cell-autonomous from non-cell-autonomous effects and initiation from progression and metastasis
Germline conditional transgenic models	• Conditional expression (tissue-specific and temporally restricted expression) of gene of interest (GOI) • The use of certain conditional promoters enables sporadic expression of GOI rather than activity in all tissue cells	• Similar disadvantages as a traditional transgenic germline model
Humanized metastasis models[234–237]	• The orthotopic injection of human tumour cells allows the evaluation of every step in metastasis • Engrafting a human tissue at the metastasis site allows the investigation of human-specific tumour–stroma interactions • Overcome limitations in transgenic mouse models that do not metastasize to bone	• Gene expression analysis of tumour versus stroma is more difficult based on limitations discussed above • Models lack an intact immune system