The Genetic Evolution of Metastasis

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ABSTRACT

Cancer is an evolutionary process that is characterized by the emergence of multiple genetically distinct populations or clones within the primary tumor. Intratumor heterogeneity provides a substrate for the selection of adaptive clones, such as those that lead to metastasis. Comparative molecular studies of primary tumors and metastases have identified distinct genomic features associated with the development of metastases. In this review, we discuss how these insights could inform clinical decision-making and uncover rational antimetastasis treatment strategies.

Introduction

Metastasis remains the primary cause of cancer morbidity and mortality. While screening programs enable early diagnosis and reduce mortality (1–3), many cancers are still diagnosed at an advanced stage (after metastases have occurred; ref. 4). With notable exceptions, treatment in this setting is not curative, highlighting the need for a better understanding of the metastatic process.

Metastatic spread involves acquisition of hallmarks of cancer (5) and additional attributes including local invasion, intravasation, and survival in the circulation, extravasation and formation of tumors at distant sites (6, 7), defined as the hallmarks of metastasis (reviewed in ref. 8).

The focus of this review is the genetic basis of metastatic evolution (9, 10) and its potential to aid patient management and drug development. Nongenetic factors that contribute to the evolution of metastasis (11–14) are beyond the scope of this review (reviewed in ref. 15).

Clonal Evolution of the Primary Tumor in Relation to Metastasis

Cancer has long been recognized as an evolutionary process in which genetic alterations provide the substrate for evolution (16). Besides selection, which leads to adaptation, a prominent evolutionary mechanism is genetic drift, reflecting random changes in mutation frequencies (17). All cells accumulate somatic alterations induced by intrinsic (e.g., spontaneous deamination of methylated cytosines, DNA oxidation) and extrinsic (e.g., tobacco smoke, ultraviolet radiation) factors (18–20). Genetic alterations that increase cell fitness (i.e., the net replication rate; ref. 21) are referred to as "driver" alterations, because they "drive" clonal expansions through constitutive mitogenic signals or avoidance of apoptosis (**Fig. 1A**). However, driver mutations, although not somatic copy number alterations (SCNA), are frequently found in clonal expansions in healthy tissues (22–28), suggesting they are not sufficient to achieve malignant transformation in isolation. Intriguingly, recent preclinical models indicate that healthy mutant populations can out-compete premalignant expansions and potentially prevent cancer (29).

Genetic alterations that occur early in tumor evolution propagate through the entire tumor cell population and are termed clonal; those arising later in tumor evolution are restricted to subpopulations of cells, and are termed subclonal (**Fig. 1A**, bottom). In the phylogenetic tree, clonal and subclonal alterations are referred to as truncal and branch, respectively (**Fig. 1B**). The existence of multiple, clonally related but genetically divergent, subclones within a tumor is termed intratumor heterogeneity (ITH; further defined in **Table 1**). Selective forces in the tumor microenvironment, for example, hypoxia (30), immune surveillance (31, 32), and therapeutic intervention (especially oncogene-directed targeted therapies; ref. 33) can profoundly alter the clonal composition of the tumor and the degree of ITH.

Three main modes of evolution have been described with respect to primary tumors: linear, branched, and punctuated (**Fig. 1C**; ref. 34). In linear evolution, genetic alterations are acquired in a stepwise fashion. A clone with increased fitness outcompetes and replaces all other clones (termed a clonal sweep), resulting in limited diversity and low ITH. In branched evolution, multiple clones of variable fitness continue to evolve in parallel, resulting in genetically divergent subpopulations and high ITH. Both linear and branched evolution reflect a gradual accumulation and selection of usually small-scale genetic alterations over time. Punctuated evolution, in contrast, is characterized by rapid acquisition of large-scale genome alterations that alter the evolutionary tempo (35). Although multiple clones may persist, the tumor mass is characterized by one dominant clone (34).

The mode of evolution in the primary tumor can impact the emergence of metastases (**Fig. 1C**). In renal cell carcinoma (RCC), for instance, punctuated evolution associates with early, widespread metastatic disease; while primary tumors characterized by branched evolution, are associated with attenuated progression and solitary or oligo metastases (36), reflecting intermediate metastatic efficiency (37). These observations highlight the importance of linking patterns of primary evolution to metastatic seeding, as a potential guide to clinical decision making.

Acquisition of Metastatic Competence: The Search for "Metastasis Genes"

The genetic basis of metastatic competence is one of the critical questions in cancer research. The clinical observation that circulating tumor cells seem to only outgrow in certain microenvironments raises

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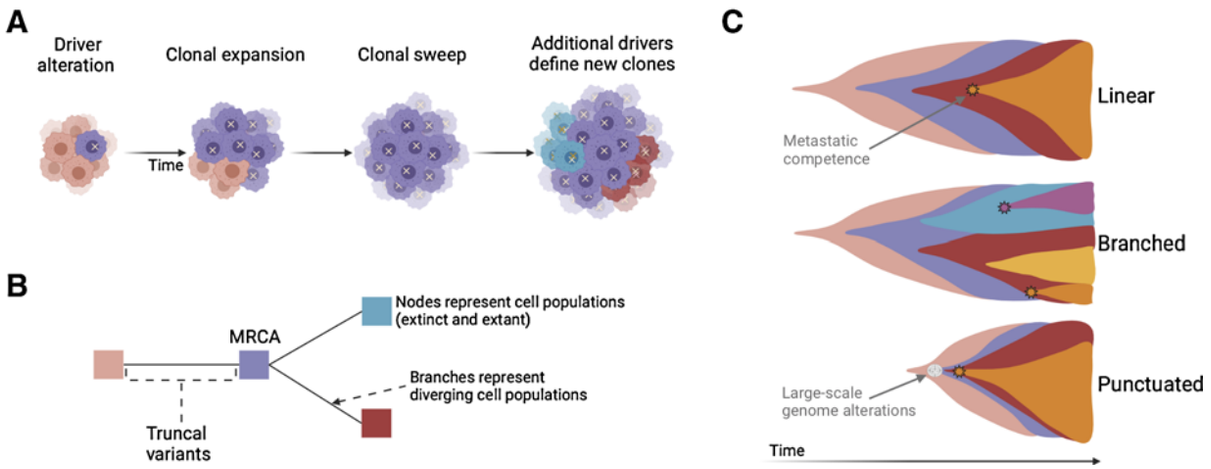

Figure 1.

A, Clonal and subclonal events. An alteration conferring a fitness advantage (indicated by a white cross) gives rise to a population of related cells (i.e., a clone, purple). At this stage, the alteration is subclonal (not present in all cells). When the subclone outcompetes others (i.e., a clonal sweep), the alteration becomes clonal. Subsequent alterations lead to genetic divergence and additional subclones, which may increase in frequency under neutral evolution or selection. **B,** Phylogenetic tree. Nodes represent clones or subclones that harbor distinct alterations. Branch length indicates genetic change between clones, often being proportional to the alterations acquired by the descendant clone. The most recent common ancestor (MRCA) of two clones is the clone that harbors all the alterations shared by the two clones. If the MRCA clone is extinct, it can be inferred from extant clones. **C,** Modes of primary tumor evolution. Clones with metastatic competence are denoted by a star. In linear evolution, clones of increased fitness outcompete and replace all others (clonal sweep), and metastatic competence may be acquired by the most advanced clone. In branched evolution, multiple clones (blue and red) continue to evolve in parallel, and metastatic competence may be acquired in minor and/or multiple clones within the primary tumor. In punctuated evolution, large-scale genome alterations radically alter the genome, resulting in early fixation of a highly fit clone. Metastatic competence may be acquired early in this context. Created with BioRender.com.

the question as to whether selection of metastatically competent clones takes place at the primary tumor site or at distant sites.

Genetic alterations associated with metastatic competence can be evaluated in primary tumors alone using clinical outcomes (i.e., metastatic relapse) as a surrogate for metastatic competence (38–40), in unmatched cohorts of primary and metastatic tumors from the same tumor subtype (41–45), and in matched primary and metastasis pairs (33, 46).

For example, the Genomics, Evidence, Neoplasia, Information, Exchange (GENIE) Consortium profiling of >1,000 primary non-small cell lung cancers (NSCLC) and unmatched metastases by MSK-IMPACT gene panel, reported a significant enrichment of *TP53* alterations in metastases (41). Further evidence of selection of *TP53* alterations during metastatic progression came from the analysis of 10,000 metastatic tumors across 62 tumor types (42). Tumor suppressor protein p53 has many roles in tumor establishment and can contribute to metastases by enhancing invasion (47), migration (48), and by inducing chromosomal instability (CIN; ref. 49). However, a comparison of whole-genome sequencing of ∼2,500 metastatic tumors from the Hartwig Medical Foundation (HMF) cohort with primary tumors from the Pan-Cancer Analysis of Whole Genomes (PCAWG) could not identify genetic alterations that were private to metastases (43, 50). Alterations in genes linked to treatment resistance such as *ESR1* and *AR* in breast and prostate cancer, respectively, are enriched in metastases (43), potentially as a consequence of selection of resistant rather than metastatic phenotypes (42, 44, 45, 51).

In the context of matched pairs, Hu and colleagues analysed 457 paired primary tumor and metastatic samples from patients with lung, colorectal, and breast cancer. Clonal driver alterations were mostly

Table 1. Main concepts.

Intratumor heterogeneity: The presence, within a single tumor, of clonally related cells or populations of cells [(sub)clones] with distinguishable genetic, epigenetic, or phenotypic features.
Metastatic competence: The ability of a cancer cell to establish metastasis.
Chromosomal instability: A form of genome instability that leads to segregation or structural abnormalities, resulting in copy number gains and losses, LOH, or aneuploidy.
Phenotype: Observable characteristics of an organism or a cell, reflecting the interaction of genetic changes with the environment.
Clade: A group of cells (or organisms) comprising a common ancestor and all its lineal descendants.
Monophyletic seeding: A mode of metastatic seeding where all metastases originate from a single clade, suggesting metastatic competence was acquired once in the evolutionary trajectory.
Polyphyletic seeding: A mode of metastatic seeding where metastases are derived from multiple clades, indicating metastatic competence was acquired multiple times.
Organotropism: Refers to the nonrandom distribution of metastases across organs resulting from the tendency of certain cancers to metastasize to specific sites.

shared between primary and untreated metastatic tumors, consistent with metastases arising from a major clone in the primary tumor. In contrast, treated metastases harbored private driver alterations, consistent with seeding from a minor subclone that evaded detection in the primary tumor or ongoing evolution at the metastatic site. These studies highlight the need for multiregional profiling of primary tumors to resolve the proposed scenarios (33).

Ultimately, the features of metastatic clones are best understood in the context of multiregional primary tumor profiling where clones that metastasize can be compared with those that do not; and metastatic clone size (whether major or minor) at the primary site can be determined. This informs an understanding of both the site and the timing of emergence of metastasis-competent clones. In the context of RCC, our group has shown that metastasizing clones exhibit high levels of chromosomal complexity, are enriched for loss of 9p and 14q (36) and are more frequently found in the center, and not the periphery of the primary tumor (52). These observations suggest that the harsh environment in the tumor center selects for SCNA-harboring clones that link to metastatic competence. Our work also highlights the critical importance of considering all classes of alterations, including copy number and structural variants. Most studies of paired primary-metastasis pairs have focused on single nucleotide variants.

In a pan-cancer analysis by Watkins and colleagues, recurrent focal subclonal SCNAs encompassing oncogenes were enriched in metastases. Some subclonal SCNAs were early events whereas others were acquired later in tumor evolution, demonstrating ongoing CIN (53). The role of SCNAs in driving metastatic risk is further demonstrated by multiple studies showing that the primary tumor SCNA burden predicts metastatic risk in breast cancer (38), uveal melanoma (39), and high-grade serous ovarian cancer (40).

A high burden of SCNAs is often a consequence of CIN, which has been shown to be strongly associated with poor clinical outcomes (54–56). CIN results in genomic DNA fragments in the cytoplasm, which can activate an innate immune response usually triggered by viral DNA: the cyclic GMP-AMP synthase–stimulator of interferon genes (cGAS–STING) pathway. In normal cells, cGAS–STING activation leads to the elimination of infected or damaged cells; in cancer cells, by contrast, it promotes invasion and metastasis (57). CIN can drive metastasis by promoting immunosuppression (reviewed in ref. 58) and epithelial-to-mesenchymal transition (EMT) in tumor cells, a dedifferentiation process in which epithelial cells lose their polarity and cell-to-cell adhesion, enhancing their migratory and invasive abilities (57, 59). Signatures of dedifferentiated states are associated with poorer outcomes in several cancer types [epithelial cancers (60), melanoma (61) and pan-cancer (62)]. This highlights the interplay between the genetic and nongenetic mechanisms that enable metastatic competence.

Whole-genome doubling (WGD) involves the duplication of the complete set of chromosomes (63). Around a third of all tumors have been shown to have undergone WGD, with significant variation by cancer type (58% of germ cell tumors vs. <5% of non-Hodgkin lymphomas). WGD is associated with adverse outcomes and is enriched in metastases, in comparison with primary tumors in NSCLC, pancreas, and prostate cancer (63). A recent report of 13 patients with lethal metastatic melanoma who underwent research postmortem showed that WGD events were more frequent in distant metastases compared with the primary tumor and locoregional metastases (64). By using long-term cultures of tetraploid colorectal cancer cells, Dewhurst and colleagues demonstrated that WGD increased tolerance to chromosomal aberrations (65). WGD could therefore favor survival and metastasis by promoting CIN, but also increased mutation tolerance and loss of neoantigens or mutations that would otherwise impede cancer progression (66).

In summary, both mutational and copy-number alterations are associated with metastasis. The strongest associations are with *TP53* mutations and CIN (67–71). The critical evidence that has emerged, however, is that metastasis-associated genomic alterations are frequently selected at the site of the primary tumor, demonstrating some overlap in the competencies required for progression at the primary site and metastatic dissemination (41).

The Clonal Relationship between Metastases

Metastatic seeding can be either mono- or polyphyletic. In monophyletic seeding, metastases are seeded from a single clade (i.e., a clone and all of its descendants). In this scenario, metastatic competence is acquired only once in the primary tumor. In polyphyletic seeding, two or more clones in the primary tumor, each derived from a different clade, acquire metastatic competence independently (**Fig. 2A**). The distinction between these modes is based on the presence or absence of a nonmetastatic primary tumor clone that shares more commonalities with a metastatic clone than with other primary tumor clones, demonstrating metastatic clones originated from multiple clades (**Fig. 2A**). Therefore, multiregional sampling and clonal deconstruction of the primary tumor is crucial to making these inferences (72). The phylogenetic relationship between metastatic clones may be misinterpreted if the primary tumor is not clonally resolved due to low sequencing depth or limited sampling.

Irrespective of their phylogenetic relationship to the primary tumor, metastatic seeding can be classified as monoclonal [i.e., from only one (sub)clone] or polyclonal (i.e., from multiple subclones), reflecting the number of primary clones detectable in the metastatic tumor. While monoclonal seeding is by definition monophyletic, polyclonal seeding can result from both mono- and polyphyletic seeding and leads to intermetastatic heterogeneity (**Fig. 2A**).

A small number of studies have described polyphyletic seeding in prostate (9), ovarian (73), renal (36), and esophageal cancer (74). Other studies did not establish the phylogenetic seeding mode, but detected polyclonal seeding in breast (75, 76) and colorectal cancer (33, 77).

Metastatic tumors themselves can be polyclonal, that is, harbor multiple subclones. Polyclonal metastases can result from multiple seeding events from the primary tumor (9, 33, 78, 79) or from metastasis-to-metastasis seeding (**Fig. 2B**), which has been shown in melanoma (80) and in pancreatic cancer (81). However, it has been suggested that other previously reported metastasis-to-metastasis seeding cases can be explained by alternative migration histories (82), highlighting the complexity of resolving tumor evolutionary histories. Cancer cells can also migrate in clusters and establish polyclonal metastases (83), a phenomenon associated with poorer prognosis in breast (84) and lung cancer (85).

The site of acquisition of metastatic competence has been described within the framework of the linear or branched models of metastatic progression. In the "linear" model, metastatic competence is gained at the primary tumor site, and there is little genetic divergence between metastatic and primary tumors (reviewed in ref. 86; **Fig. 2C**, top). In the parallel progression model, the clone destined to form metastases departs the primary tumor before acquisition of metastatic competence, and evolves at a distant site, in "parallel" to the primary tumor (**Fig. 2C**, bottom). This results in greater genetic divergence between the primary tumor and the metastases.

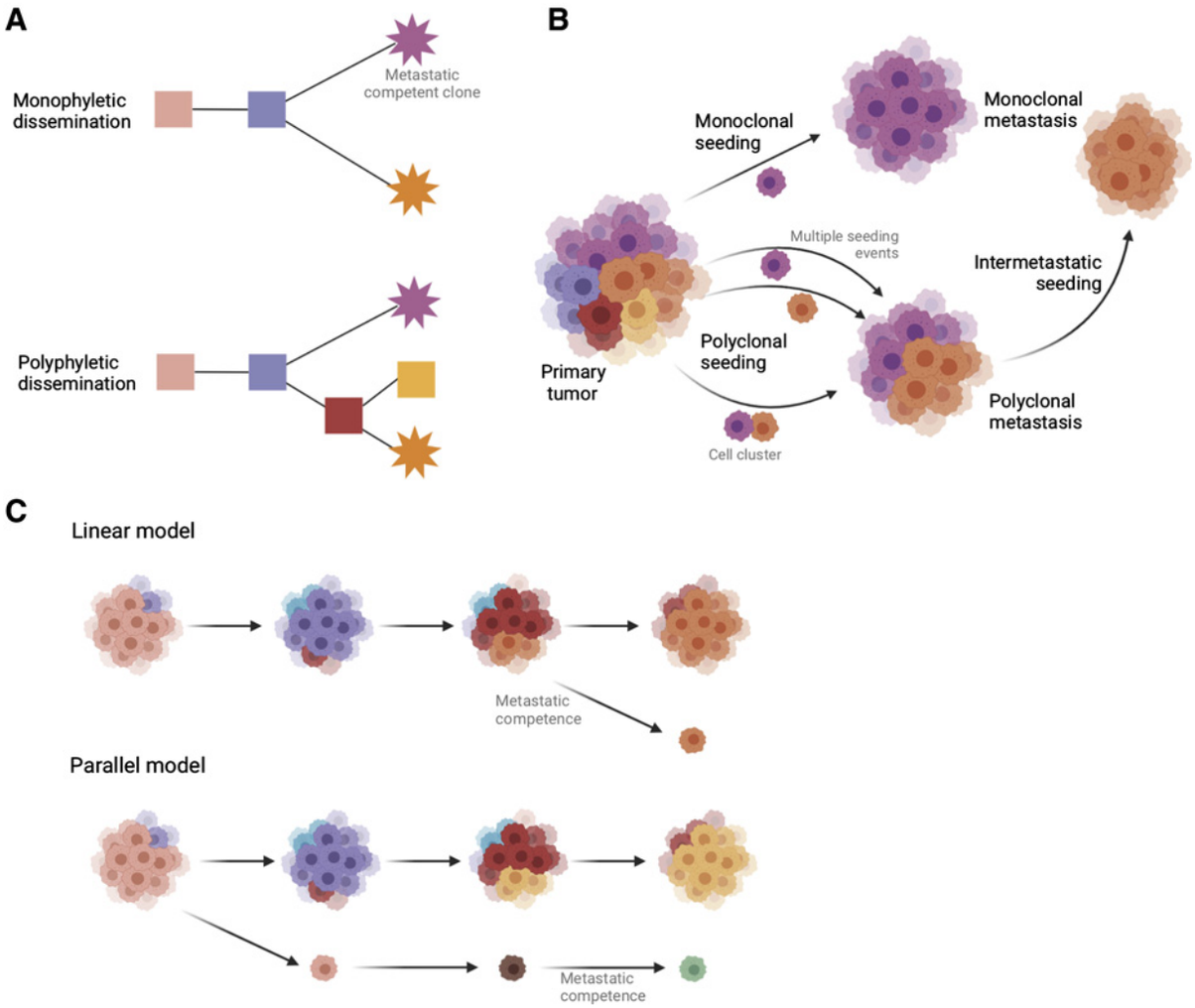

Figure 2.

A, Phylogenetic modes of metastatic dissemination. Phylogenetic trees represent the relationships between primary tumor clones, including metastatic seeding clones (represented by stars). In this example, the most recent common ancestor (MRCA) of all metastatic cells is represented by the purple square. Monophyletic dissemination occurs when all descendants of the MRCA seed metastases. When a nonmetastatic seeding clone (yellow square) is more closely related to a metastatic clone than to others, the metastatic seeding mode is polyphyletic. **B,** Metastatic seeding clonality. When a single metastatic competent clone (burgundy) seeds a metastasis, the metastatic seeding mode is monoclonal. Polyclonal seeding occurs when multiple primary tumor clones (burgundy and orange) seed metastases, which can result from multiple separate seeding events or from cells migrating together in clusters. **C,** Models of metastatic competence evolution. In the linear model, metastatic competence is acquired at the primary tumor site (orange cells). Metastatic and primary tumors are more similar in this scenario, as they share most of their evolutionary history. In the parallel model, tumor cells that are not yet able to seed metastases leave the primary tumor (pink). These cells stay dormant and acquire metastatic competence (green) in a protected niche. In this model, a higher genomic divergence between primary and metastatic tumors is expected, as they share a shorter portion of their evolutionary history. Created with BioRender.com.

As discussed, metastasis-associated genomic alterations are frequently present in the primary tumor, and these observations provide support for the linear model of metastatic progression. Nevertheless, these inferences can be significantly impacted by sampling bias. If the metastasis originates in a minor subclone in the primary tumor, it can evade detection, leading to an erroneous conclusion of a parallel model of metastatic competence. Furthermore, missing the nonmetastatic clones in the primary tumor that support the polyphyletic origin of metastatic clones will lead to monophyletic seeding inferences. Once again, multiregional sampling, liquid biopsies (87) and representative profiling (88, 89) are potential strategies to mitigate these biases.

Timing of Metastatic Progression and Organotropism: Implications for Clinical Detection and Management

The proclivity to metastasize may be determined in the early stages of neoplastic progression, for example, through punctuated evolution, with patients presenting with *de novo* metastatic disease. In clinical practice, metastatic relapse is sometimes detected decades after removal of the primary tumor (90, 91), a phenomenon stereotypically observed in breast cancer (92) and melanoma (93). As metastases stem from the primary tumor prior to surgical removal, this suggests

that disseminated tumor cells may not progress to immediately detectable metastases. Disseminated cells either acquire metastatic competence subsequent to leaving the primary tumor (parallel model) or are already metastasis-competent (linear model) but remain dormant.

Dormant cancer cells were originally described as "malignant cells [that], although remaining alive in the tissues for relatively long periods, show no evidence of multiplications during this time, yet retain all their former and vigorous capacity to multiply" (94). Dormancy has been interpreted variably and dormant cells have been referred to as "drug-tolerant cells", "persister cells", "metastasis-initiating cells", and "latency-competent cells". The biological characteristics of dormancy are reviewed in detail elsewhere (95).

The variable time to relapse between patients likely reflects the presence of occult fully competent metastases (early relapse) and time taken to reactivation of dormant cells is the result of cell-intrinsic and microenvironmental factors in the target organ (late relapse, usually >10 years; ref. 96). Various microenvironmental factors have been implicated in the exit from dormancy including recruitment and activation of osteoclasts (97, 98), secretion of proangiogenic factors (99), and immune evasion (100, 101). Understanding the cues for entering and exiting dormancy raises the possibility of therapies aimed at extending the dormant phase (when cancer eradication is not achievable; ref. 102).

In addition to temporal patterns of metastasis, cancers are also distinguished by stereotypical patterns of spread to different organs. Most clinically detectable distant metastases are found in liver, lung, bone, and brain, whereas organs such as kidney, heart, and stomach are less commonly colonized (103). Cancer subtype–specific patterns are notable: breast and prostate cancer typically associate with bone metastases (104), whereas uveal melanoma has predilection for liver metastases (105).

Although James Ewing proposed that cancer cells were directed by the lymphatic and circulatory systems (106), Stephen Paget noted the disconnect between blood supply and frequency of metastasis in certain organs, conceptualized as the "seed and soil hypothesis" where the cancer cells are the "seeds" and the specific organ microenvironment the "soil" (107), implying that selective pressures are tissue context dependent. To date, there is limited data on the genetic bases of organotropism. However, our group has shown that pancreatic metastases from RCC, compared with metastases to other organ sites, are characterized by low levels of SCNAs and absence of loss of 9p and 14q (which are frequently selected at other metastatic sites; ref. 36). These findings are in keeping with the very indolent nature of RCC metastases to the pancreas (36).

Brain metastases are of particular interest as they are associated with the most morbidity and mortality in patients with cancer. Brastianos and colleagues analyzed 86 matched primary and metastasis pairs (lung, breast, and renal cell carcinoma) and found potentially actionable genetic alterations in brain metastases that were not detected in the primary tumor in about 50% of patients (108). These observations are consistent with brain metastases either arising from a minor subclone in the primary tumor or evolving at the metastatic site. In the context of NSCLC, Shih and colleagues identified enrichment of *MYC*, *YAP1*, and *MMP13* amplification and *CDKN2A/B* deletion in brain metastases and showed that *MYC*, *YAP1*, and *MMP13* overexpression promoted brain metastases in patient-derived xenografts (109).

Implications of Metastasis for Treatment Resistance

Most metastatic cancers have been considered incurable. In the context of oncogene-directed targeted therapies, durable responses have been observed in a small subset of patients (110). However, in most patients, resistance develops as a result of selection of preexisting resistant clones. In *EGFR*-mutant NSCLC, Offin and colleagues found a negative association between tumor mutation burden (TMB) and clinical benefit from EGFR inhibitors (111), that is, higher TMB may be correlated with a greater number of preexisting resistant subclones. In the setting of immune checkpoint blockade, in contrast, high TMB associates with higher likelihood of treatment response due to the resultant high burden of neoantigens (112). The advent of immune checkpoint blockade has brought about durable disease control (and possible cure) for some patients with metastatic melanoma (113), NSCLC, and renal cell carcinoma (114). Nevertheless, treatment resistance remains a critical challenge in oncology. Intrinsic or primary resistance implies the presence of preexisting highly prevalent resistance-conferring alteration. Acquired resistance presents as progression after an initial response, suggesting that the selective pressure of therapy selects a preexisting, likely minor, subclone carrying the resistance-conferring alteration(s) (115). Indeed, mathematical models predict that most radiographically detectable metastases already carry at least 10 resistant subclones (116), therefore the likelihood of resistance scales up with increasing burden of metastatic disease.

Treatment resistance can occur through a variety of genetic mechanisms. In the context of BRAF–MEK inhibition, resistance can be driven by alterations of the target oncogene (e.g., *BRAF* amplification; ref. 117), reactivation of the MAPK pathway (e.g., *NRAS* or *MEK* mutations; ref. 118), or activation of an alternative signaling pathway (e.g., *PTEN* loss enhancing PI3K signaling; refs. 119, 120). Notably, in a patient with breast cancer who became resistant to a PI3K inhibitor, Juric and colleagues detected 6 different *PTEN* alterations across 10 metastases, showing how distinct resistance can evolve in parallel converging on the same mechanism under the selective pressure of oncogene-directed targeted therapy (121).

Given the relationship between disease burden and the likelihood of treatment resistance (primary or secondary), (neo)adjuvant treatments, are a compelling approach to reduce the risk of recurrence after surgical resection (122). It is notable that when applied in the adjuvant setting, targeted therapy can potentially result in cure of a proportion of patients, suggesting that resistant clones were eliminated (123). This shows elegantly that population size (microscopic vs. macroscopic disease) impacts whether certain populations become fixed and expand or become vulnerable to stochastic perturbations (124, 125). One of the challenges of adjuvant therapy is the inability to measure treatment effect in real-time (akin to minimal residual disease in hematologic malignancies; ref. 126). However, detection of circulating tumor cells or cell-free tumor DNA may serve as a surrogate marker for adjuvant treatment effect (127), similar to circulating tumor DNA (86, 128, 129).

In the context of resistance to immune checkpoint blockade, several immune evasion mechanisms have been identified. Alterations in antigen-presenting machinery genes and concurrent loss of heterozygosity (LOH) have been detected in melanoma (130, 131). LOH of the human leukocyte antigen (*HLA*) locus is enriched in lung cancer metastases (132). HLA-LOH improves survival prediction over TMB alone in lung cancer (133) and is a prognostic biomarker in triple-negative breast cancer (134). HLA-LOH is frequently a subclonal event, highlighting further the role of CIN (132) in metastatic disease.

The prominent role of CIN in metastasis and treatment resistance (38, 56, 57, 135) makes it an attractive therapeutic target. Two strategies have been theorized: the first strategy seeks to correct the segregation defects to minimize ITH and tumor adaptability. A second, fundamentally different, strategy involves CIN-inducing therapies that would exacerbate CIN to such a degree that chromosomal missegregation defects would be incompatible with cancer cell survival. A clear downside would be systemic exposure of healthy cells (135–139). Another compelling target to prevent or treat metastases would be p53, specifically the restoration of functional p53 (140, 141). Several pharmacologic approaches in clinical development include targeting of MDM2 (or p53–MDM2 binding) although the clinical implementation remains challenging and combination approaches are likely needed given the presence of other genetic alterations (reviewed in ref. 142).

Brain metastases are a specific area of unmet need and based on the findings of the genetic association of brain metastases including *CDKN2A/B* deletions (109) clinical trials are underway with CDK pathway inhibitors palbociclib and abemaciclib in patients with brain metastases across different cancers (NCT02896335, NCT02308020; ref. 143). This is an example of how the understanding of genetic underpinnings of metastases can translate into potential therapeutic options for patients.

Conclusion

Metastasis remains the primary cause of cancer morbidity and mortality despite major improvements in cancer treatments.

Primary and metastatic tumors have been compared in multiple studies to better define the genetic basis of metastatic progression. Although certain individual genetic features have been implicated in metastasis development, none are metastasis-exclusive. These observations suggest that alterations that confer fitness advantages in early tumor evolution also contribute to metastatic dissemination (as in the case of *TP53*), and that nongenetic mechanisms play an essential role in metastatic progression.

CIN and *TP53* are well established as gatekeepers in the genetic evolution towards metastasis across cancer types. The identification of numerous SCNAs associated with metastases across cancer types supports the notion that larger-scale genetic alterations may be crucial in the establishment of clones with metastatic competence. These observations are conceivable given that SCNAs lead to changes in the expression of hundreds of genes, compared to mutations in a single gene. Direct targeting of mechanisms underpinning CIN and aneuploidy remains challenging, however, individual targets such as the CDK pathway in brain metastases with *CDKN2A/B* deletions demonstrates how understanding the genetics of metastases can enhance therapeutic development.

The roadmap to understanding metastatic disease will require biobanking initiatives and large consortia (e.g., GENIE, HMF) to support comparative studies and increase statistical power, whole-genome sequencing efforts [e.g., PCAWG, Genomics England (GEL)] to interrogate the noncoding genome, and postmortem studies (e.g., PEACE NCT03004755, CASCADE; ref. 144) and multiregional paired studies (e.g., TRACERx initiatives; refs. 36, 56) to understand the patterns of metastatic spread.

Authors' Disclosures

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