PERSPECTIVE

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Selection and adaptation during metastatic cancer progression

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Cancer is often regarded as a process of asexual evolution driven by genomic and genetic instability. Mutation, selection and adaptation are by convention thought to occur primarily within, and to a lesser degree outside, the primary tumour. However, disseminated cancer cells that remain after 'curative' surgery exhibit extreme genomic heterogeneity before the manifestation of metastasis. This heterogeneity is later reduced by selected clonal expansion, suggesting that the disseminated cells had yet to acquire key traits of fully malignant cells. Abrogation of the cells' progression outside the primary tumour implies new challenges and opportunities for diagnosis and adjuvant therapies.

Concern that progress in treating patients with epithelium- or neuroectoderm-derived cancers is simply too slow is increasing. Notwithstanding the great enthusiasm about the potential for improvements to cancer treatment that was evoked by cancer genome projects and the cancer stem-cell concept in the past decade, targeted therapies currently prolong the lives of patients with metastasis by only a few weeks or months[1–4]. Moreover, they often have little or no benefit when given to at-risk patients with no metastasis, that is, as adjuvant therapy[5–7]. In light of these observations and the multilevel complexity and heterogeneity of systemic cancer in particular, focusing merely on individual genetic alterations and corresponding targeted therapies is unlikely to be the most promising approach. We have learned and will probably continue to learn that many, if not all, genes have roles in certain subtypes of some cancers for particular steps in the metastatic cascade in some models. But can we use this knowledge to improve patient treatment?

Since its description by Cairns and Nowell[8,9], the evolutionary concept of cancer has become widely accepted; this concept has recently been updated in an excellent review[10], and the molecular mechanisms of metastasis have been reviewed and conceptualized in detail[11–13]. One limitation of the literature on metastasis is that findings are readily included without acknowledgement of the relevance and limitations of patient- and model-derived data. However, mutation, selection and adaptation are linked to the environment of the evolving cancer cell; therefore, differences in these are likely to matter. This Perspective concentrates on human metastasis by attempting to derive an evolutionary concept of cancer based on patient-derived molecular and clinical data, focusing on the early evolution of systemic cancer. However, in doing so, it comes with important caveats, the most relevant being the paucity of information about the phenotypes and early interactions of cancer cells that spread to distant sites. Once these cells form a metastatic colony, the tumour-generated environments of metastases (whether in the lungs, liver or elsewhere) resemble, at least morphologically, those of primary tumours. Consequently, the dynamics of cellular adaptation resulting from heritable genetic or epigenetic changes or phenotypic plasticity (see the Review by Meacham and Morrison on page 328) on are poorly understood in patients. This Perspective summarizes what can be deduced from currently available, mostly genomic, data and may stimulate research into the emergence of cellular cancer phenotypes that cause lethal disease.

Hallmarks of benign lesions and malignant cancer

It might be helpful to recall the basic characteristics of cancer before considering systemic progression in detail. Malignant epithelial tumours are able to form vascularized colonies (metastases) at different sites in the body, whereas benign tumours cannot. By definition, then, cancers can invade and metastasize. However, the molecular differences between benign and malignant tumours are woefully understudied. Apart from circumstantial investigations of their genetics, we have a relatively poor molecular appreciation of benign tumours.

Benign tumours in principle can be divided into two groups: those with a statistical association with progressing to cancer and those without. In many instances, the risk of progression is not known, because proving the transition from benign to malignant is not easy. A benign lesion under the pathologist's microscope will never progress to cancer, so the lesion's history and fate can only be linked by association with cancer detected simultaneously or post hoc in the same patient. It is illuminating to consider examples in which malignant conversion is thought not to occur, in which a biological continuum of benign and malignant cancers is debatable and in which it is widely accepted.

The most insightful and comprehensive studies yet on benign lesions that apparently have no risk of malignant conversion have come from Hafner, Real and their teams. From human benign skin tumours, such as seborrhoeic keratosis one of the most common[14,15], they identified multiple mutations of oncogenes such as *FGFR3*, *PIK3CA*, *KRAS*, *EGFR*, *HRAS* and *AKT1* (listed in order of decreasing frequency), demonstrated activation of the mutated signalling pathways and showed that no senescence program is activated by these mutations[15]. Of note, FGFR3 kinase domain mutations leading to the highest constitutive kinase activity[16] are more frequent in benign seborrhoeic keratosis than in urothelial carcinoma, paradoxically suggesting that just benign lesions may harbour mutations with strong oncogenic potential. Although only a limited number of candidate oncogenes were tested, combinations (most frequently (42%) *FGFR3* and *PIK3CA*) were uncovered, and it is reasonable to expect that genome-wide analyses will reveal additional combinations. There were no signs of genome instability as tested by array comparative genomic hybridization (CGH)[15] (Fig. 1a).

In breast cancer, the debate on the existence of a biological continuum from benign to malignant lesions is ongoing[17,18]. The true relationship of ductal hyperplasia, atypical hyperplasia and ductal carcinoma *in situ* (DCIS) is unclear. This may partially reflect the relative difficulty of

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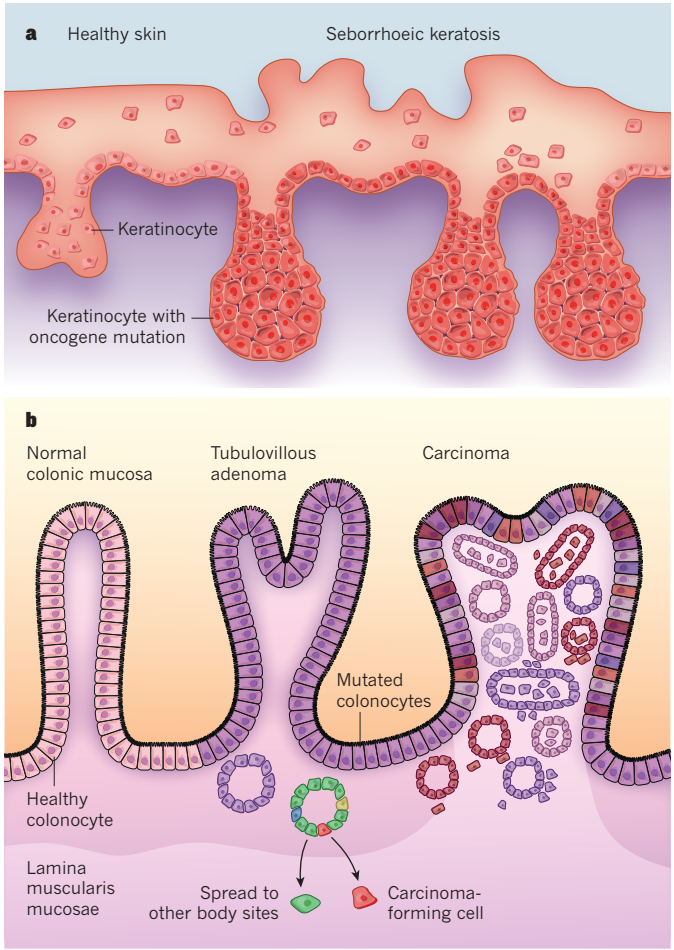

Figure 1 | Mutations in benign tumours. Proving the transition from benign lesion to malignancy is not easy. It can be helpful to consider examples in which conversion is thought not to occur and in which the continuum between benign and malignant cells is accepted. **a**, Seborrhoeic keratosis is a benign skin tumour in which proliferative cells harbour various mutated oncogenes such as *FGFR3* and *PIK3CA*, but in which neither conversion to malignancy nor genome instability is observed. **b**, Clonal diversity is generated during the adenoma–carcinoma sequence of colonic dysplasias. Genome diversity (shown by the differently coloured cells) seems to emerge at the transition from dysplasia to carcinoma. Cells may migrate beyond the lamina muscularis mucosae and form a carcinoma or spread to distant sites in the body. Note, however, that unlike *in situ* carcinomas of the breast, cancer cell dissemination has not yet been formally shown for colonic dysplasias.

morphological assessment compared with, for example, lesions in the colon. The few data there are available for benign proliferative breast disease suggest that although hyperplasia may display limited allelic losses, DCIS is principally distinguished by the emergence of genome instability, as delineated by fluorescence *in situ* hybridization (FISH) and CGH[18,19].

The best-studied example of a putative biological benign–malignant continuum is the adenoma–carcinoma sequence in the colon[20], and clinical procedures have been developed to link the detection of adenomas with prevention or early diagnosis of colorectal cancer[21]. Malignant lesions are characterized by invasion of the lamina muscularis mucosae and beyond. Initial analysis of small adenomas, large adenomas and carcinomas has uncovered an accumulation of genetic alterations paralleling clinical progression[20]. The two main classes of benign lesion are conventional adenomas (comprising tubular, tubulovillous and villous adenomas) and serrated polyps (comprising hyperplastic polyps, traditional serrate adenomas, sessile serrated adenomas and mixed hyperplastic adenomatous polyps)[22,23]. Molecular analyses have focused on three types of event: point mutations, chromosomal rearrangements and CpG methylation changes[24]. For conventional adenomas, high-grade dysplasia is related to larger adenoma size and a villous component and is an important risk factor for metastasizing cancer. In molecular terms, high-grade dysplasia correlates with the global frequency of allelic imbalances (Fig. 1b), whereas 5′-methylcytosine changes and *KRAS* mutations can also be found in low-grade dysplasias[25]. For serrated polyps[23], the hyperplastic polyp accounts for 25–30% of resected large intestinal polyps and is the most frequent category. It has an insignificant malignant potential (under current guidelines, no additional surveillance is required[21]) but frequent *BRAF* or *KRAS* mutations[22,23]. Hyperplastic polyps have a normal karyotype with a small subset displaying simple chromosomal aberrations[26].

In summary, benign lesions share DNA methylation changes, restricted loss of heterogeneity and multiple oncogenic mutations with malignant lesions, but they are insufficient to predispose lesions to invasion and metastasis. Rather, the difference between benign and malignant lesions is apparently associated with the emergence of genome instability. I therefore suggest that the cardinal hallmark of cancer is an ongoing production of genomic diversity, which enables gene networks to self-organize and individual cells to progress towards metastasis in a process of clonal evolution[10,27] (see Review by Swanton and colleagues on page 338). The extent of genome diversity varies but is usually substantial in solid epithelial cancers and melanoma[28,29]. To elaborate this concept, important data are still needed, including direct genome-wide cell-by-cell comparison between genomic diversity in benign lesions and early invasive cancers and careful analysis of global epigenetic change, because epigenetic diversity may also drive clonal evolution and influence genomic stability[10].

Cellular diversity and metastasis

The importance of diversity in malignancy is further supported by a study in which Maley and colleagues demonstrated that a high index of clonal diversity predicts progression from a pre-malignant condition (in this case the progression of dysplastic Barrett's oesophagus) to an aggressive cancer (adenocarcinoma of the oesophagus)[30]. Such a direct clinical link between diversity and invasive or metastasizing cancers is lacking for other cancers. However, in another study, whereas clonal diversity between DCIS and invasive breast cancer was similar, breast cancer subtypes displayed different degrees of diversity[31]. The less aggressive (that is, less metastatic[32]) luminal A cancers comprised a few dominant cancer cell populations, whereas the more aggressive basal-like and HER2$^+$ cancers contained a wider array of less abundant tumour cell types[31] more frequently, when specific genomic loci were investigated by FISH.

If cellular diversity correlates with benign and malignant states, and if increasing diversity indices are associated with the propensity to seed colonies at ectopic sites, what is the role of diversity in metastasis? An obvious hypothesis is that the generation of variant cells increases the probability that some are sufficiently equipped to successfully migrate to, colonize and survive in distant sites. Typical metastatic sites, such as bone marrow, lung, liver and brain, form heterogeneous environments, which are more likely to provide hostile niches for intruding cancer cells than permissive ones. Disseminating cell population size, the proportion of cells able to self-renew, their genotypic diversity and phenotypic plasticity (the ability of one genotype to elicit more than one phenotype in different environments) are all determinants of metastatic success.

What follows is a discussion of disseminated cancer cell (DCC) adaptation to ectopic sites (those outside the site of origin). This, it is argued, is an evolutionary process engendering fitness to generate a metastatic colony, requiring researchers to consider whether selection occurs within or outside the primary tumour.

DCC genomes in early and advanced systemic cancer

Cancer cells often start to disseminate on generation of genetic diversity at the primary site. Early dissemination can be deduced from disease course and patient-derived data[33–35] (reviewed in ref. 35). Direct

evidence is growing that presumptive pre-invasive lesions such as *in situ* carcinomas can seed cancer cells[36–38] (Fig. 1b).

One might imagine that dissemination forms a bottleneck that restrains genome diversity found among individual DCCs. If this is so, we have not yet identified the underlying rules, suggesting that these are either too subtle or complex, or that dissemination is linked to a specific phenotype, not genotype. Note, however, that we are lacking sufficient data about the phenotype of DCCs. Genomic cell-by-cell analysis of DCCs isolated from bone marrow or lymph nodes of patients with carcinoma but without overt metastases revealed substantial heterogeneity during occult (undetectable by clinical imaging) systemic spread before manifestation of clinical metastasis[39–43] (Fig. 2a, c). When more than one cell was analysed, sibling cells from an individual patient rarely shared multiple chromosomal aberrations, but frequently possessed distinctive genomes[39,41,43]. Thus, genome diversity is evident in early systemic cancer. DCCs from bone marrow and lymph nodes have also been shown to harbour characteristically different chromosomal aberrations[42].

In all cancers investigated, DCC genomes become highly similar once metastases have clinically manifested, suggesting that expansion of an aggressive clone parallels the development of generalized, incurable disease[39,43] (Fig. 2a, b). The pattern of chromosomal aberrations in late-stage disease seems relatively stable, because, even after several cycles of high-dose chemotherapy, a shared common baseline of gains and losses can be identified[39] (Fig. 2c).

Macroevolution and microevolution of DCC genomes

DCCs in patients with metastases present a recurrent pattern of genomic and genetic changes referred to here as an attractor[44]. This preferred genomic state is also seen in circulating tumour cells (CTCs) isolated from patients with metastasis. The few CTC data so far mostly describe genome alterations of pooled CTCs[45,46], but detection of typical late-stage alterations is evident. Putative attractor genomes of DCCs and CTCs from patients with manifest metastasis resemble those found in corresponding primary tumours[39,41,43,47].

The genomic difference between DCCs isolated from patients with no distant cancer spread (M0 stage) and patients with cancer spread (M1 stage) is striking and fundamental (Fig. 2). Without knowing the disease stage of a patient with breast cancer, it is possible to predict with 85% accuracy whether the patient has manifest metastasis[41] by determining the chromosomal aberrations of single cytokeratin-positive cells isolated from the bone marrow. This is not possible with genomic information from the primary tumour. For the remainder of the Perspective, I will therefore differentiate between DCC-M0-like and M1/primary tumour (PT)-like genomes.

DCC-M0-like genome data are limited and await comprehensive investigation with modern sequencing technologies. So far, the number of chromosomal changes has been found to be lower than in M1/PT-like genomes[39,41,43]; typical chromosomal aberrations expected for the cancer under consideration are mostly absent[41,43]; evidence for telomere crisis is lacking as usually gains or losses of whole chromosomes, but less frequently chromosome breaks, occur[41]; point mutations characteristic of the cancer under consideration are mostly absent[39]; and subchromosomal losses and gains often precede the accumulation of gains or losses of whole chromosomes[40] (Fig. 2a). Thus, DCCs isolated mostly from the bone marrow of M0-stage patients seem to disseminate in a genomically 'immature' state in which they have not yet acquired typical mutations in oncogenes and tumour suppressor genes or copy number changes, and in some cases may not be immortal — as defined by the absence of signs of passage through telomere crisis. The genomes of such cells will be referred to as DCC-M0-like, although individual DCCs in a patient may exhibit these five traits of malignancy to different degrees.

In contrast to DCC-M0-like genomes, M1/PT-like genome changes match the chromosomal signatures of their associated cancers. Evidence that typical changes resemble attractor states in the somatic evolution of a cancer comes from many studies such as cytogenetic and molecular genetic analyses that reveal cancers acquire chromosomal aberrations non-randomly, with preferred patterns of gain and loss for each cancer[29,48]. For example, in breast cancer, typical M1/PT-like aberrations include losses of chromosome 8p and 17p and gains of 8q and 17q, which are mostly absent in DCC-M0-like genomes. But clearly, similarities between cancers of one type from different patients do not imply the cancers are clonal and, accordingly, the attainment of similar attractors does not imply clonal descent, for example, of metastasis from a specific region of a primary tumour. Furthermore, sequencing studies revealing genomic heterogeneity in primary tumours and metastases suggest that several attractor states may exist for a given cancer[49].

It may be helpful to differentiate between macroevolution and microevolution in the somatic evolution of DCCs. Here, macroevolution refers to the transition of relatively normal DCC-M0-like genomes to M1/PT-like genomes (Fig. 2b), and microevolution to the ongoing evolution of DCCs with M1/PT-like genomes. Macroevolution results from evolutionary shifts[50], and it is difficult with our current understanding to explain the transition from DCC-M0-like to M1/PT-like genomes without such a shift. Evolutionary shifts could be induced by processes such as telomere crisis or the inactivation of an important tumour suppressor (for example, p53). Atypical chromosomal changes found in the diverse

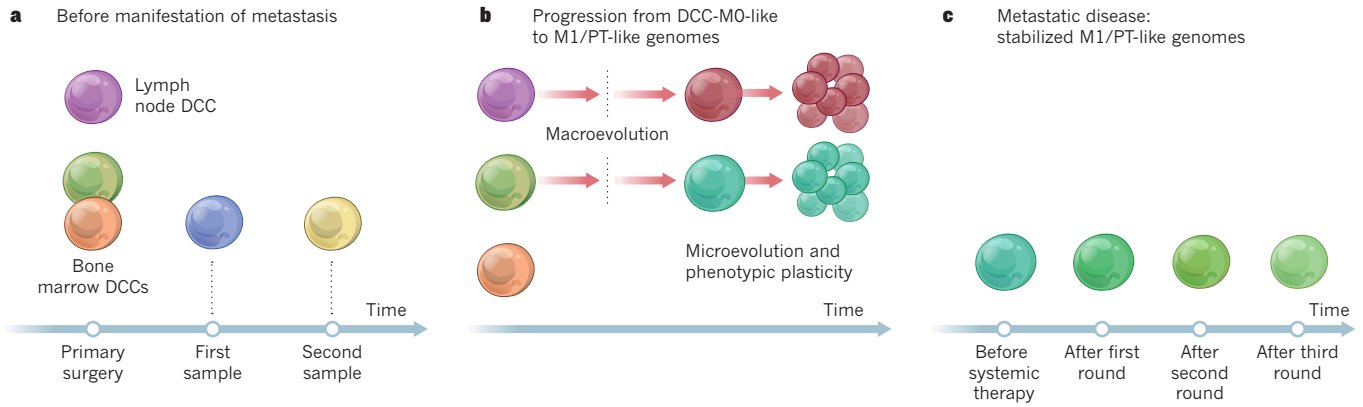

Figure 2 | Macroevolution and microevolution of breast cancer genomes. **a**, Heterogeneity of genomes of disseminated cancer cells (DCCs) before manifestation of metastasis (DCC-M0-like). Bone marrow and lymph node DCCs have different genomes, as do DCCs isolated at different time points. DCC-M0-like genomes have few and atypical copy number alterations, no signs of telomere crisis, subchromosomal loss that occurs before whole chromosome change and few point mutations. **b**, Progression of DCC-M0-like genomes to the genomes of cells that are found in manifest metastases/primary tumours (M1/PT-like). Cells with DCC-M0-like genomes must undergo macroevolution to M1/PT-like genomes. Additional layers of diversity are generated by clonal expansion, microevolution and phenotypic plasticity. **c**, Relative stability of M1/PT-like genomes after manifestation of metastasis. Even over the course of several rounds of systemic treatment genomes display similarity. M1/PT-like genomes have many and typical copy number alterations, chromosomal breaks, typical point mutations and evidence of clonal expansion.

DCC-M0-like genomes may increase the fitness of individual cells in a specific micro-environment. Chromosomal changes alter the expression of many genes[51], potentiating progression to M1/PT-like genomes. Once M1/PT-like attractor states have been attained, the expanding metastasis-forming cell populations may evolve gradually. Microevolution is this gradual evolution within a population of cells that have reached a genomic attractor state.

Plausibility and potential relevance of macroevolution

DCC macroevolution is unstudied but may be important for several reasons. First, unlike benign tumours or putative precursor lesions, DCC-M0-like genomes belong to a DCC population directly linked to cancer through an originating primary tumour. In its defining ability to spread is the potential seed of metastasis. Because DCCs represent the earliest stages of cancer, they may allow more clinically relevant investigation of carcinogenesis than benign or putative precursor lesions, for which transition to malignancy is unpredictable. Second, high-resolution DCC-M0-like genome analysis may help to identify early changes and therapy targets[40]. Third, the transition from DCC-M0-like genomes to the attractor state of M1/PT-like genomes may form a significant hurdle, which could be raised by new modes of therapeutic intervention, or may suggest new barriers that could be induced artificially.

Although the occurrence of early dissemination and the existence of DCCs with immature DCC-M0-like genomes are increasingly accepted, the relevance of these cells as seeds of metastasis is questioned and negated[12,13]. It may be impossible to prove the cells' relevance in patients because the founder cell of a metastasis may never be identified and DCC-M0-like genomes may be outcompeted and disappear once M1/PT-like genomes emerge. Support for the cells' role in progression of human cancer will come from a consistent and coherent clinical picture if it exists. However, circumstantial evidence supporting the argument for DCC-M0-like genomes already exists.

Rare detection of M1/PT-like DCCs in M0-stage patients

A major argument in favour of a role for DCCs with DCC-M0-like genomes is that it is exceptional for DCCs with M1/PT-like genomes to be detected in bone marrow at the time of curative surgery. DCCs comprise all cancer cells that spread before the removal of the primary tumour. If cells with M1/PT-like genomes form the predominant cell population in the primary tumour at the time of sampling, why are they rare? Technically, detection of DCCs depends on the sensitivity and specificity of available markers. The two most commonly used markers for epithelium-derived DCCs, glandular cytokeratins (mostly keratin 8, 18 and 19) and EpCAM (originally called 17-1A antigen) were introduced by Riethmüller and Schlimok in the 1980s (ref. 52). These markers are suited to the detection of epithelial DCCs in tissues such as blood, bone marrow and lymph nodes because these tissues lack epithelial cells. However, expression of both markers can be low or absent in epithelial cells, so DCCs may escape detection — the reasons for this downregulation or loss are not understood. Both markers are expressed in early embryonic development[53] or human embryonic stem cells[54,55] in normal and malignant cells[56,57] with differentiated, progenitor-cell or stem-cell features[58–61]. Detection of cells with either marker in the blood, bone marrow or lymph node tissue of cancer patients is associated with poor prognosis[62]; however, there are typically only 1–10 positive cells for every 10^6 normal bone marrow or lymph node cells in less than 50% of M0-stage patients. In summary, despite limitations that are inherent to all markers, there is good reason to think cytokeratin and EpCAM expression capture relevant DCCs. Moreover, because both DCCs from metastatic and non-metastatic patients have been identified with the same markers[39–43], it would be inconsistent and unfounded to suggest that the markers regularly miss cells with M1/PT-like genomes in M0-stage patients but detect them in M1-stage patients.

Biologically, the explanation for M1/PT-like genome rarity is based on studies involving thousands of patients[37,62,63]. Increasing DCC counts did not correlate with tumour size, even though there may have been several hundred-fold more cancer cells in large primary tumours than in small ones, suggesting that constantly disseminating cancer cells in bone marrow do not simply accumulate over years of primary tumour growth[37,62,63]. There are three possible explanations for this. First, there is no increased number of DCCs in bone marrow over time because dissemination and death of DCCs in bone marrow are balanced. Second, early cancers are more capable than late cancers of generating cells that are adapted to disseminate, integrate and survive

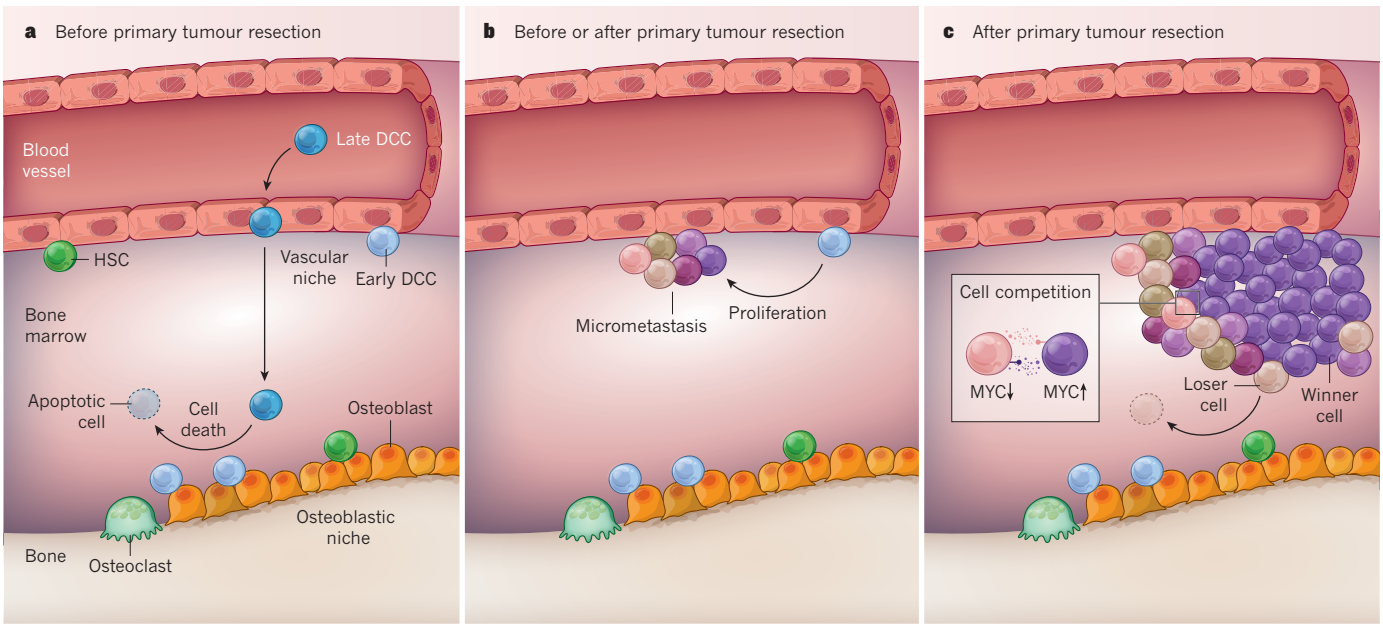

Figure 3 | Early steps of metastasis at ectopic sites in bone marrow. **a**, Before removal of the primary tumour, cell competition for occupancy of vascular and osteoblastic niches may favour early disseminated cancer cells (DCCs). Both niches may be used by DCCs; however, late DCCs may find that the niches are occupied by early DCCs and die as a result of apoptosis. **b**, Before or after surgery, DCCs must proliferate to form a micrometastasis, which is essential for selective adaptation and progression towards cells with M1/PT-like genomes. **c**, During micrometastasis formation, cells may engage in competition whereby secreted factors allow the recognition of fitness differences between cells. Cells with high levels of MYC, which have higher fitness (winner cells), induce apoptosis in cells with low MYC, which have lower fitness (loser cells).

in bone marrow. Third, there are a limited number of niches in bone marrow (and probably in other organs), so these are unavailable to cells that disseminate later.

Balanced dissemination and death, can be discarded at least for prostate cancer, as the percentage of DCC-positive bone marrow samples is constant (around 20%) before surgery and more than a decade post-surgery in non-progressing patients[43]. All patients had undergone radical prostatectomy, so no later DCCs could be seeded, suggesting that DCCs are long-lived but — contrary to the study's finding — would have continued to accumulate where the primary tumour remained with the ability to seed. Decreasing dissemination or integration and the limited number of niches, probably both contribute. Cells representing the predominant clone of the primary tumour at resection may rarely disseminate or compete with early DCCs or other cells that occupy the niche and seem to be maladapted to displace early-DCCs from their niche (Fig. 3a). Experimentally, prostate cancer cells compete with haematopoietic stem cells (HSCs) for the endosteal niche in bone marrow, and direct competition assays have revealed that the number of niches is limited and that malignant and normal cells have different (lower and higher, respectively) affinities for them[64]. If these findings can be extended to patients, they may further explain why bone-seeking cancers (such as breast and prostate) are not initially associated with much higher numbers of DCCs than cancers that exhibit bone metastasis less frequently (such as colon cancer)[62], as early seeding rates would be sufficient to fill the available niches.

Asynchronous and independent evolution of metastases

For all carcinomas, patients initially present with local lymph node metastases in the absence of distant metastasis far more frequently than vice versa. This is reflected in the classic International Union Against Cancer (UICC) staging system, for which stage III represents local lymph node metastasis without distant metastasis and stage IV represents disease with distant metastasis, regardless of lymph node status. It would therefore be expected, that more DCCs from lymph nodes progress further at the time of curative surgery and contain M1/PT-like genomes more frequently, particularly when micrometastases are present, than do DCCs from bone marrow. This is indeed the case[42], suggesting that metastatic colony growth is generally associated with evolution towards M1/PT-like genomes — this is, however, asynchronous. Despite their evolutionary head start, lymph node micrometastases are unlikely sources of distant metastases, as evidenced by many clinical studies demonstrating that removal of the micrometastases has no effect on distant metastases[65–69], supporting the idea that DCCs at different sites evolve into M1/PT-like cells independently.

Genomic comparisons of primary tumours and metastases

Cells with DCC-M0-like genomes progressing towards M1/PT-like genomes become similar to the primary tumour — but not identical. Genetic disparity and clonal divergence within the primary tumour, and between primary tumours and paired metastases were observed even before the advent of next-generation sequencing technologies (studies up to 2009 have been summarized in ref. 70), but have generally been corroborated since[49,71–74]. Disparity is seen for all types of genetic alteration, including point mutations[49,72–74], rearrangement breakpoints[75], areas of chromothripsis[71] (an extensive genome scrambling[76]), collectively supporting ongoing genomic evolution at metastatic sites. However, other studies have found higher similarity between primary tumours and paired metastases or relapsing cancers[77–79]. Explanations for why some primary tumours and metastases are more closely related than others are manifold. Clinical details, such as the time interval between primary tumour resection and biopsy or surgery of the metastasis, or the type and extent of systemic treatment are often omitted. Cancers with explosive growth at primary and distant sites might result from aggressive, highly similar clones[78], whereas metastases arising after long latency periods may differ extensively[73]. Toxic chemotherapy drugs may homogenize cancer cell populations at primary and distant sites in stage IV patients who have not undergone surgery, by generating cells with high phenotypic plasticity that are able to colonize the whole body (Fig. 4). This highlights the need for clinical information, without which studies[79] are difficult to evaluate.

Time considerations

The latency period that is needed to initiate systemic cancer from DCCs with DCC-M0-like genomes would provide a much better explanation of the time courses of patients' diseases than the assumption that the most aggressive fully malignant clones initiate the metastases. Most human cancers double their tumour volume within 60–250 days, but prostate cancer has a tumour volume doubling time (TVDT) of 700 days[35]. Consequently, human cancers can often take several years to diagnose. The fastest growing 5% of breast cancers can reach 1–2 cm within 1 year, whereas the slowest growing 5% reach this size within about 5 decades[80,81]. The time course of metastasis suggests that metastases have similar growth rates to primary tumours[35,80]. By contrast, the TVDT of xenograft models using cell lines, such as MDA-MB-231, is less than 1 day and that of transgenic or knockout mouse models between 3 and 12 days. Since the former start with M1/PT-like genomes and the latter are initiated by transgenes or loss of tumour suppressor genes that result in strong oncogenic potential (changes that are rarely detected in DCC-M0-like genomes), a major proportion of the natural evolution from a normal cell is abrogated, which may at least partially explain DCCs' accelerated growth in models. In short, xenograft and many transgenic cancer models fail to model one or more key phases of human cancer.

DCC microevolution in advanced metastatic disease

Once metastasis is diagnosed, DCCs and CTCs display M1/PT-like genomes, which display less heterogeneity by CGH analysis. In metastatic disease, microevolution operates as in MDA-MB-231, which have been found to display rather stable M1/PT-like genomes despite selection at different sites, including bone marrow, lung and brain[82–84]. Chromosome profiles for MDA-MB-231 subpopulations that metastasized to different organs were similar despite marked differences in expression patterns of metastasis-associated genes and metastatic activities and the exhibition of site-specific gene expression signatures[83]. Cells with M1/PT-like genomes, once they have undergone several rounds of selection, seem to develop high phenotypic plasticity, enabling them to resist various types of selection pressure. Therefore, metastasis models based on cell lines model metastatic processes that occur in end-stage metastatic patients.

Factors promoting metastatic progression

If the progression of DCCs with DCC-M0-like genomes is important for human metastasis and if the progression provides a time window for intervention, what are the underlying mechanisms? Answering this may enable new paths for intervention. Currently, there are no data but by analogy to carcinogenesis and *in vitro* selection experiments cell competition and micro-environmental factors may have major roles.

The recognition of DCCs with 'incompletely mutated' DCC-M0-like genomes is reminiscent of studies (for example, see ref. 85) that have attempted to induce cancer from normal cells. These experiments uncovered the need for initiating (genotoxic and mutating) as well as promoting (non-genotoxic and non-carcinogenic) agents[85] to generate skin cancer. Promoting agents, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), can exert their effects long after carcinogens have initiated DNA lesions and been removed[86]. *In vitro* selection experiments have shown that cell density and cell–cell contact are crucial for full spontaneous transformation of initiated (mutated) cells[87–89]. Therefore, single DCCs must first start proliferation (possibly out of dormancy) to form a micrometastasis (Fig. 3b). Then, the driving force for progression of DCC-M0-like genomes towards M1/PT-like genomes may likewise be selection and cell competition (Fig. 3c). Cell competition is a selection process in which 'loser' cells with low fitness are eliminated (for

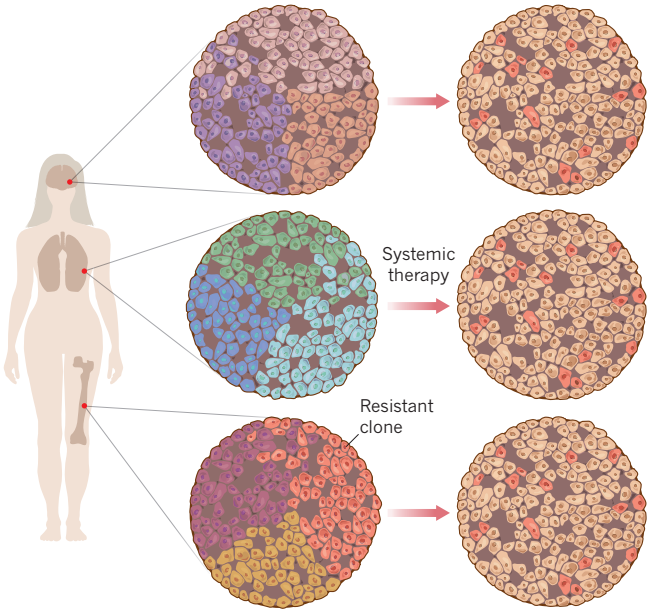

Figure 4 | Heterogeneity of metastasis and homogenization by systemic therapies. Independent somatic evolution, cell competition and various systemic and site-specific selection pressures (such as the extracellular matrix, hypoxia and immunosurveillance) generate metastatic diversity. Although systemic therapy may eliminate cells, resistant clones may overgrow after selection by therapy. These resistant cells may have been present as a minority at one metastatic site before therapy and then seeded other sites in the body (shown here) or they may have already been present at various metastatic sites. The extent of an homogenizing effect by repeated rounds of systemic therapies on resistant cancer cells is currently unknown. Cells that survive multiple rounds may display high phenotypic plasticity for growth throughout the body.

example, through induction of apoptosis) by 'winner' cells with higher fitness[90,91]. During development, we know of two types of competition: adhesion-based competition for niche occupancy and direct cell–cell comparison of metabolic status[90]. In both cases, cell fitness is sensed and communicated, and variation of both could play a part in metastasis. The relevance of cell competition to cancer is increasingly recognized thanks to the work of Moreno[92] and colleagues, and it is noteworthy that competition involves pathways known to have a role in cancer, including MYC, TP53, WNT and JAK–STAT pathways[91]. Selection would produce winning cells with alterations in these pathways and indeed amplification of *MYC* on chromosome 8q is clearly associated with M1/PT-like genomes in breast and prostate cancer DCCs[41,43], as is loss of 17p and *TP53* mutations[39,41]. Whether competition for anatomical niches has a role in systemic cancer is still debated, but data from animal models suggest that it does[64]. If cell competition is needed to select for higher cell fitness, metastatic progression may occur only if DCCs expand and engage in competition (Fig. 3b, c), because M0-like DCCs left the primary tumour early and thereby avoided cell competition at the primary site.

The tumour-promoting effect of TPA may be due at least partly to altering the micro-environment by inducing massive inflammation[93]. The role of micro-environmental changes (see the Review by Junttila and de Sauvage on page 346) in promoting the transition from DCC-M0-like to M1/PT-like genomes may therefore be of particular interest. Experimental primary tumours may suppress outgrowth of micrometastases by preventing the induction of angiogenesis[94] or by imposing DCC dormancy through secretion of *meta*- and *ortho*-tyrosine[95]. They may also stimulate metastatic outgrowth by the secretion of factors, such as osteopontin or S100A8 that recruit bone-marrow-derived cells to the incipient or established site of metastasis[96–98]. Primary tumour exosome secretions may even evoke the 'education' of tissues such as bone marrow resulting in the long-lasting promotion of metastasis[99]. The local environment at the distant site, such as sprouting neovasculature, may also trigger outgrowth of dormant DCCs[100]. Although many of these studies have been performed using cell lines with M1/PT-like genomes, there is no reason to think that indirect metastasis-promoting effects through engagement of supportive cell populations would not also affect DCCs with DCC-M0-like genomes.

Future directions

The concept presented in this Perspective remains to be tested for a broad range of cancer types. Most data are available for breast and prostate cancer and interesting differences between these cancers and more aggressive cancers have been highlighted[101]. The concept should also be scrutinized for its relevance to diagnosis and therapy. It predicts that certain characteristics of M1/PT-like genomes present in DCCs before manifestation of metastasis are predictive of patients with shortened relapse-free survival. It further stresses the need to determine directly the molecular characteristics of DCCs and CTCs so that the evolution of the disease can be monitored at all stages and the appropriate (targeted) therapies selected. Basic research will be needed to address the phenotype of cancer cells adapting to different selective conditions before and after the cancer cells have induced their own tumour-like environment. For therapy development, retarding cellular macroevolution may be much more effective than attempts to kill M1/PT-like cells, and require a change to current adjuvant therapy strategies. Notably, initial attempts have been made specifically to target aneuploid genomes with drugs[102] that are arguably more effective in DCCs with DCC-M0-like genomes that have not yet reached the M1/PT-like attractor state because such drugs target existing aneuploidy-associated stresses[51]. Adjuvant therapy clearly requires substantially different strategies to abrogate metastatic disease[7], including the design of clinical trials[103] (see Review by Siu and colleagues on page 355). Although evolution of different clones at different sites is unlikely to be synchronized, this should not stop us from exploring options to suppress tumour-promoting effects. Analysis of large clinical and epidemiological data sets for the temporal and spatial distribution of metastases suggests that initially (without interference from genotoxic therapies) neither distant nor lymphatic metastases are themselves able to metastasize[65,104]. Therefore, if we successfully manage to retard progression of DCCs with M0-like genomes to M1/PT-like genomes by non-genotoxic means, more patients may present with surgically manageable metastasis (if they present with any at all), which could provide clinicians with a second chance to completely ablate the patient's disease. ■

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