REVIEW

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Clonal evolution in cancer

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Cancers evolve by a reiterative process of clonal expansion, genetic diversification and clonal selection within the adaptive landscapes of tissue ecosystems. The dynamics are complex, with highly variable patterns of genetic diversity and resulting clonal architecture. Therapeutic intervention may destroy cancer clones and erode their habitats, but it can also inadvertently provide a potent selective pressure for the expansion of resistant variants. The inherently Darwinian character of cancer is the primary reason for this therapeutic failure, but it may also hold the key to more effective control.

Cancer is a major cause of death throughout the world and, despite an extraordinary amount of effort and money spent, the eradication or control of advanced disease has not been achieved[1]. Although we have a much greater understanding of cancer biology and genetics[2], translation into clinical practice needs to allow for the cellular complexity of the disease and its dynamic, evolutionary characteristics. These features provide both barriers to, and opportunities for, successful treatment.

In 1976, Peter Nowell[3] published a landmark perspective on cancer as an evolutionary process that is driven by stepwise, somatic-cell mutations with sequential, subclonal selection. This is a parallel to Darwinian natural selection, with cancer clones as the equivalent of asexually reproducing, unicellular quasi-species. Modern cancer biology and genomics have validated cancer as a complex, Darwinian, adaptive system[4,5] (Box 1 and Supplementary Information).

Cancer-clone evolution takes place within tissue ecosystem habitats. These habitats have evolved over a billion years to optimize multicellular function but restrain clonal expansion of renegade cells. However, the resilience of multicellular and long-lived animals depends on the phenotypic properties that, if not tightly regulated, drive or sustain malignancy: that is, cellular self-renewal and stabilization of telomeres, which allow extensive proliferation, angiogenesis, cell migration and invasion[6].

The long time period usually required for cancer symptoms to emerge and the complexity of the resultant mutations is, in part, a reflection of the sequential and random searches for phenotypic solutions to constraints from the micro-environment. The evolutionary progression of cancer is usually stalled or aborted, as shown by the high frequency of clinically covert premalignant lesions[7–9]. Cancer-suppressive mechanisms relegate most cancers to old age, when they have little effect on the reproductive fitness of their hosts.

Limited resources, environment architecture and other constraints of the micro-environment limit the size of solid tumours at every stage of their progression. Even advanced malignancies can show Gompertzian growth[10] — the cancer cell doubling time (around 1–2 days) is orders of magnitude faster than tumour doubling time (around 60–200 days)[10] — implying that the vast majority of cancer cells either die before they can divide[11] or are kept from dividing by the tumour micro-environment. Thus, natural selection in tumours, in the same way as selection in organisms, takes place through competition for space and resources.

Oncologists change cancer-clone dynamics by introducing a potent source of artificial selection in the form of drugs or radiation, but evolutionary principles still apply. Usually, treatment will result in massive cell death, which provides a selective pressure for the proliferation of variant cells that resist treatment (the mechanisms for this are discussed later). Furthermore, many cancer therapeutics are genotoxic; cells surviving treatment, which could then go on to regenerate the cancer, may have mutated further, resulting in cells with improved fitness and malignant potential.

The tools of and insights from evolutionary biology and ecology can therefore be applied to the dynamics of cancer before and after treatment to explain the modest returns from cancer therapy. We show that cancer is an inherently evolutionary process and suggest alternative strategies for effective control.

Mutational drivers and clonal dynamics

The basic principle of a Darwinian evolutionary system is the purposeless genetic variation of reproductive individuals who are united by common descent, together with natural selection of the fittest variants. Cancer is a clear example of such a system. Most mutational processes have a bias at the DNA sequence level. The particular mutational spectra in a cancer cell can be a reflection of error-prone repair processes or associated with a genotoxic exposure (for example, cigarette carcinogens, ultraviolet light and chemotherapeutic drugs[2]). The patterns of genetic instability (chromosomal or microsatellite) in cancer cells may reflect exposure to, and the selective pressure exerted by, some classes of chemical carcinogens[2]. Nevertheless, for the functions encoded in genes, mutagenic processes are essentially blind or non-purposeful (with the exception of intrinsic mutagenic or recombinatorial enzymes preferentially targeting lymphoid immunoglobin or T-cell receptor genes[12]). The recurrent, mutation-endowed fitness traits in cancer reflect the potent impact clonal selection can have.

Clones evolve through the interaction of selectively advantageous 'driver' lesions, selectively neutral 'passenger' lesions and deleterious lesions (a 'hitchhiker' mutation in evolutionary biology is equivalent to a passenger mutation in cancer biology). In addition, 'mutator' lesions increase the rate of other genetic changes[13,14], and micro-environmental[15] changes alter the fitness effects of those lesions. The identification of driver lesions is supported by the independent observation that these lesions occur more frequently in multiple neoplasms than would be expected in the normal background mutation rate, that they are associated with clonal expansions[16,17] and from the type of mutation seen (missense, nonsense, frameshift, splice site, phosphorylation sites and double deletions)[18–20], particularly if the gene involved has a known role in cellular processes relevant to oncogenesis. The evidence gained from genetic studies

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of human tumours should be corroborated with functional tests and animal models. Passenger lesion status can also be ambiguous or context-dependent: for example, cases of monoallelic loss that only impact on function when the second allele of the same gene is lost, mutations that only cause a phenotypic effect when another gene locus also mutates, or cases in which the mutants are functionally relevant only in the context of therapeutic responses involving that gene.

Only a few studies have attempted to quantify the selective advantage provided by driver mutations. Bozic *et al.*[21] (using a non-spatial population genetics model of sequential, exponential clonal expansion) derived a formula for the proportion of expected neutral passenger mutations versus the proportion of selectively advantageous driver mutations as a function of the selective advantage of the driver mutations. By fitting this equation to glioblastoma and pancreatic cancer resequencing data, the authors estimated that driver mutations gave an average fitness advantage of only 0.4% (ref. 21). To measure the mutant clone selective advantage directly would require longitudinal samples of a neoplasm and estimation of the clone sizes at each time point.

The dynamics of somatic evolution depend on the interaction of mutation rate and clonal expansion. Mutation rate varies substantially between different genomic regions[22] and between different types of abnormality (for example, single-base sequence changes versus balanced chromosomal rearrangements and gene fusions), and mutation rates will increase by the instigation of genetic instability[23–25]. The rate of epigenetic change has been estimated to be orders of magnitude higher than that of genetic change[26], and could be a major determinant of clonal evolution. Natural selection affects epigenetic variation within neoplasms[27], because epigenetic changes are inherited at cell division and can affect cell phenotypes. Evolutionary biology tools to address many of these mutation rate complexities exist (see Supplementary Information), but these remain under used in cancer biology[28]. The traditional model of clonal evolution suggests that a series of clonal expansions grows to dominate the neoplasm ('selective sweeps')[16,21,29], but this can occur only if the time to the next driver mutation is longer than the time required for a clone to sweep through the neoplasm. In addition, if the second mutation occurs in a competitor clone, the expansion of both clones is restrained by mutual competition (known as clonal interference)[30]. Given the large population size and high mutation rate typical of neoplasms, clonal competition is probably common[31,32]. This issue is best addressed by serial sampling, and limited data suggest that parallel clonal expansions occur before subclones begin to dominate in early cancer development[33–35]. Initial evidence indicates that large clonal expansions after cell transformation are rare[26]. Direct evidence, from serial sampling of oncogenic mutations in advanced disease[36], metastasis[37] or post-chemotherapy relapses (see Supplementary Information), indicates selective sweeps originate from pre-existing genetic variants or subclones.

Punctuated equilibrium versus gradualism

The argument of gradualism versus punctuated equilibrium[38] (a longstanding debate in species evolution) has recently emerged in the consideration of the clonal evolution of neoplasms. It is unknown whether malignant clones, with their markedly altered genomes, evolve gradually through a sequence of genetic alterations and clonal expansions; accumulate many lesions over time in a rare, undetected subclone that finally appears in a clonal expansion; or have a few, large-scale punctuated changes, possibly prompted by an acute insult or a single, catastrophic mitotic event that generates multiple lesions across the genome (or on a single chromosome, known as chromothripsis)[39]. Evidence of tens of non-synonymous mutations in cancers was interpreted under the assumption that they were generated by tens of clonal expansions[29]. Reconstruction of genealogies of neoplastic clones, based on genetic heterogeneity within neoplasms, suggests that clones with ancestral genomes are not driven to extinction by later clonal expansions[31–33], which allows the history of a neoplasm to be revealed. Breast cancer data[32] have shown that clones with intermediate genotypes are difficult to detect; each clone generates a cloud of genetic neutral or non-viable subclone variants around it. A study of B-cell chronic lymphocytic leukaemia[40] suggests that intermediate clones can be detected, but at a frequency of <0.001, which was below the detection threshold of the breast cancer study[32]. Intermediate clones may be rare because they have had limited potential to expand or because they were once common but were outcompeted by more recent clones.

The frequency of premalignant clonal lesions (or carcinoma *in situ*) substantially exceeds clinical cancer rates[7–9]. This, as well as cancer dormancy[41] and genetic reconstitution of clonal histories[37], indicates that cancer clones have long periods of stasis. However, cancer-clone evolution probably passes a point of no return, possibly at the metastatic growth stage. If unlimited proliferative capacity is guaranteed by telomere stabilization[25], then clonal expansion is stopped only when the size threatens the life of the patient. When provided (albeit rarely) with the routes for dissemination and immunoselection, cancer cells can have a parasite-like immortality and can re-establish themselves in other individuals[6,42,43].

BOX 1

Cancer as a complex system

- Cancers exist in a variety of taxonomic quasi-classes, genera, species, characterized by divergent cells of origin and mutational spectra. Each cancer is unique.
- Cancers evolve over a variable time frame (anywhere from 1 to 50 years), and the clonal structure, genotype and phenotype can shift over time in each patient. Each cancer is, in effect, multiple different (subclonal) cancers that occupy overlapping or distinct tissue habitats.
- The number of mutations in a cancer can vary from a handful (10–20) to (the more usual) hundreds or thousands. The great majority are passengers, and a modest, but undefined, number are functionally relevant drivers. The mutational processes are very diverse.
- Cancers acquire, through mutational and epigenetic changes, a variety of phenotypic traits that compound to allow territorial expansion, by proliferative self-renewal, migration and invasion — properties that are cruical to normal developmental, physiological and repair processes.
- Advanced, disseminated or very malignant cancers seem to be almost uniquely competent to evade therapy.
- Most, if not all, of this complexity can be explained by classical evolutionary principles.

The cancer ecosystem

Tissue ecosystems provide the venue and determinants for fitness selection (the adaptive landscape[44]). Tissue micro-environments are complex, dynamic states with multiple components that can influence cancer-clone evolution (Fig. 1). For example, transforming growth factor-β is a cancer-ecosystem regulatory molecule[45]. Other cellular and cytokine components of inflammatory lesions are potent and common modulators of the cancer-cell ecosystem[25].

The interaction between cancer cells and their tissue habitats is reciprocal. Cancer cells can remodel tissue micro-environments and specialized niches to their competitive advantage[46]. Cancer-clone expansion is controlled by architectural constraints or barriers, such as sequestration of stem cells into crypts in the gastrointestinal tract[47]

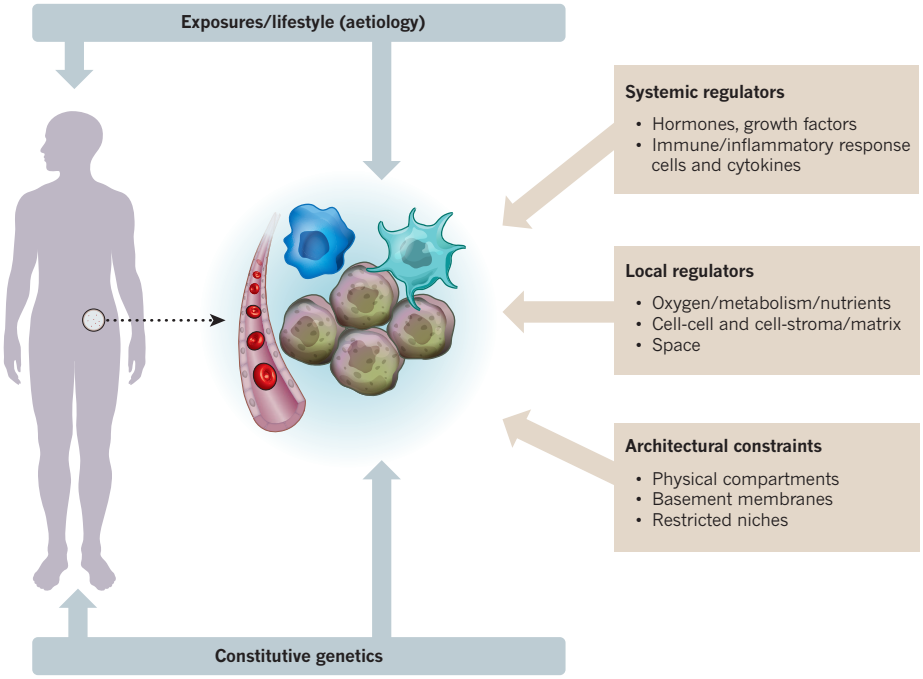

Figure 1 | The complexity of tissue ecosystems. Exposure, the constitutive genetics of the host cells, systemic regulators, local regulators and architectural constraints all impinge on the evolution of somatic cells.

and the need for external signals for proliferation and cell survival. However, some micro-environmental components can promote neoplastic cells; for example, infiltrating macrophages and neovascularization, in response to anoxia, can support neoplastic cell survival and proliferation. Mathematical modelling shows cancer-clone evolutionary selection for more robust or malignant phenotypes is less likely in more stable or homogeneous micro-environments[48]. Spatial heterogeneity of resources in the primary tumour selects for cell migration and emigration, which may explain why there is selection for metastasis[49]. Preclinical models have suggested that normalizing the resources across the primary tumour can suppress metastasis[50]. As clones and subclones expand, migrant cells invade new habitats within and between tissues, in which they experience new selective pressures that can cause further cancer-cell diversity. This malignant feature, and its associated morbidity, characterizes end-stage cancer.

Cancer-cell habitats are not closed systems. The tissue ecosystem, in addition to regulation by systemic factors (such as nutrients and hormones) or invasion by inflammatory or endothelial cells, is modified by external factors. As well as the tissue site, the ecosystem for each cancer includes environmental, lifestyle and associated aetiological exposure of the patient. Genotoxic exposure (such as, cigarette carcinogens or ultraviolet light), infection, and long-term dietary and exercise habits that affect calorie, hormone or inflammation levels can have a profound effect on the tissue micro-environments, as well as directly on cancer cells (Fig. 1). These factors are the aetiological link to the initiation or progression of cancer, and without such modulating exposure, the risk of cancer-clone initiation and evolution would be reduced.

Cancer-tissue ecosystems can be radically altered after chemotherapy or radiotherapy. Most cancer cells may be decimated, but the remodelled landscape creates new selective pressures, resources and opportunities that may allow pre-existing variant cancer cells that survived treatment to emerge. Crucially, stroma or specialized habitat niches may protect cancer cells against the therapy[51].

Cancer genomics and clonal architecture

Cancer-genome sequencing, facilitated by the introduction of second-generation whole-genome sequencing, has provided further insight into the complexity of the genetics and evolutionary biology of cancer cells[2]. In most cases, transformation and metastases are probably clonal[2] because they are derived from single cells; therefore, the identification of the mutations present in all of the cells of a tumour can help to reconstruct the genotype of the founder cell. These founder events limit the genetic and clonal complexity of tumours. We already had a long list of recurring driver mutations (with gain or loss of function) as a result of the fine mapping of chromosomal breaks, candidate gene sequencing and functional screening of bulk samples from tumours. However, the use of genomic screens has demonstrated the scale of cancer-genome complexity. Individual cancers can contain hundreds, or tens of thousands, of mutations and chromosomal alterations[2]. The great majority of these are assumed to be neutral mutations arising from genetic instability. Chromosomal instability (amplifications, deletions, translocations and other structural changes) is a common feature, but it is not clear whether there is an increased rate of simple base-pair mutations in cancer[2,21,23,52]. Evolutionarily neutral alterations are thought to register in the screens because they hitchhike on clonal expansions that are driven by selectively advantageous alterations or by drift. In addition, data have confirmed that each cancer in each patient has an individually unique genomic profile. It is possible that cancer cells need only a modest number of phenotypic traits to deal with all of the constraints and evolve into a fully malignant or metastatic tumour[25], but the genomics data suggest that this can be achieved by an almost infinite variety of evolutionary trajectories and with multiple different combinations of driver mutations[44].

Paradoxically, genome profiles underestimate complexity. So far, they have been mostly one-off snapshots from a single sample at a single diagnostic time point. We know that serial or parallel sampling using more conventional genetic analysis uncovers genetic diversity within a tumour. Whole-genome sequencing of paired primary tumours versus metastatic samples has so far been limited, but it has revealed that individual metastatic lesions are clonal in origin and genetically unique, yet have a clonal ancestry traceable to the primary tumour[2]. 'The genome' description is perhaps also misleading because genetic variants are identified in 5–50% of reads, which suggests subclonal distribution of most mutations[53], but the segregation pattern of mutations within subclones is lost when DNA is extracted from the total cell population. This is important if patient-specific genomic profiles are to provide a platform for selecting therapeutic targets. Arguably, subclonal genetic diversity is key to the success or failure of therapy. This is a considerable challenge, technically and bioinformatically, in cancer genomics and will require deep sequencing[40]

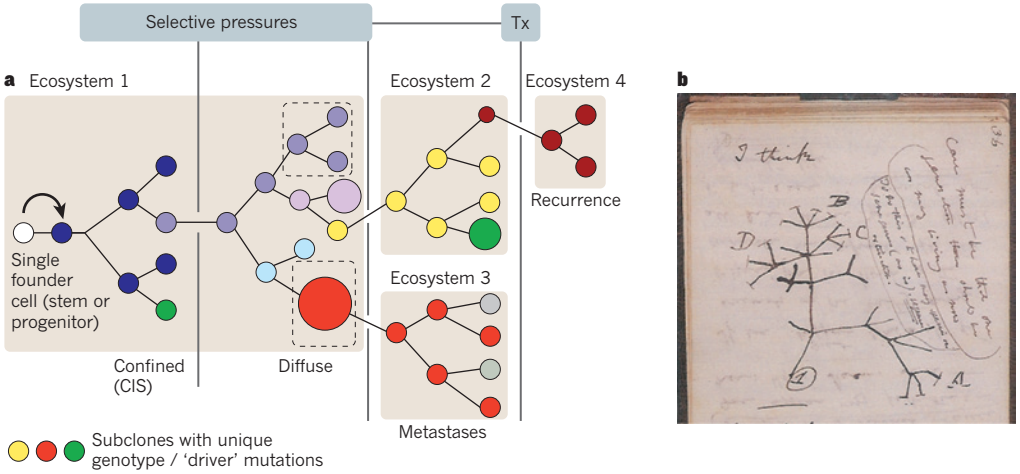

Figure 2 | The branching architecture of evolution. **a**, Cancer clones. Selective pressures allow some mutant subclones to expand while others become extinct or remain dormant. Vertical lines represents restraints or selective pressures. This is a representative pattern for common, solid cancers; as recognized by Nowell[3], leukaemic clones may expand over a shorter time frame (years versus decades), and be subject to fewer restraints and mutational events. Ecosystems 1–4 (boxes) represent the different tissue ecosystems or habitats. Smaller boxes within Ecosystem 1 represent localized habitats or niches. Each differently coloured circle represents a genetically distinct subclone. Metastatic subclones can branch off into different time points in the sequence from either minor or major clones in the primary tumour. Tx, therapy. CIS, carcinoma *in situ*. **b**, Darwin's branching evolutionary tree of speciation from his 1837 notebook.

and investigation of the genomes of single cells for patterns of segregation of mutations to understand the genetic diversity within neoplasms and how this changes in response to interventions.

Subclonal segregation of mutations and clonal architecture

The classic model of clonal evolution suggests there is a sequential acquisition of mutations with concomitant, successive subclonal dominance or selective sweeps. Histopathological evidence of disease progression (adenoma, carcinoma and metastases) supports this model. At each stage of this evolution, individual cells and their progeny (subclones) compete for space and resources. Multiplexed, single-cell mutational analysis (ideally in serial samples) is the most appropriate way to examine clonal architecture. So far, there are only a few examples of this[10,32,33], but they have provided evidence of the complex pattern of subclonal segregation of mutations — consistent with Nowell's model. The large amount of data from tissue sections, small biopsies and, more recently, single-cell analysis[33] is evidence that the evolutionary trajectories are complex and branching, exactly as Nowell proposed and in parallel with Darwin's iconic evolutionary speciation tree (Fig. 2). Attempts to simplify this complex system into a linear sequence of mutational events on the basis of cross-sectional data have probably been misleading[54]. However, by comparing the mutational genomes of the subclones, it is possible to discover their evolutionary or ancestral relationships, as well as the order of events during the development of that neoplasm[32,33,37,53,54]. Clonal evolution from common ancestral cancer cells is demonstrated in identical twins with concordant acute leukaemia[55,56], in metastatic lesions[2,10] and, by inference, in some cases of bilateral testicular cancer[57] (Fig. 3). In this context, divergent cancer-clone genotypes and phenotypes correspond to allopatric speciation in separate natural habitats (for example, Darwin's finches on the Galapagos Islands[58]).

Profiles of subclones within a neoplasm can be used to determine 'molecular clocks' that can then be linked to time events in the history of the neoplasm. For example, DNA methylation changes and base-pair mutations have been used to infer clonal expansion dynamics[26] and the time between initiation, invasion and metastasis[17,37,52]. It is even possible to determine the relative timing of events during progression from a single sample, based on deep sequencing[59].

Subclones may be mixed together within the primary tissue[37,60], but given their single-cell origin and bifurcating pathways, it is not surprising that they can also occupy distinctive territories[35,37,61,62] (Fig. 4a). Cancer-clone evolution involves contemporaneous subclones with distinctive mutational and phenotypic profiles that may be territorially segregated, which has considerable practical implications for diagnosis, prognosis and targeted therapy based on biopsy sampling[63]. It remains unclear whether all subclonal diversification reflects the impact of driver mutations and selective advantage, or is also the result of genetic drift of selectively neutral mutations or even epigenetic alterations. The level of diversity within the subclonal structure can be measured[35,64,65] and has been shown to be a robust biomarker for predicting progression to malignancy in Barrett's oesophagus[65]. It is also associated with the tumour stage and subtype of breast cancer[64].

Units of selection and cancer stem cells

Evolutionary theory suggests that natural selection operates in any system that has components with varying reproductive potential[4]. In

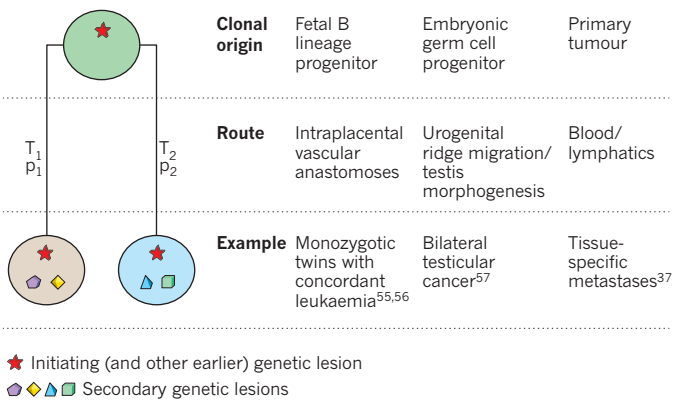

Figure 3 | Divergent (branching) clonal evolution of cancer with topographical separation. In each example, a clonal (single cell) ancestry is indicated by a shared acquired mutation (for example, *ETV6–RUNX1* fusion for leukaemias and *KIT* mutation for testicular cancers). The time at which the two subclones evolve (T_1 and T_2) can be temporarily synchronous or develop several years apart[37,55–57]. The probabilities of subclones emerging as shown are independent and different (p_1 and p_2). In most cases (90% for monozygotic twins), only one twin develops overt leukaemia. The penetrance of bilateral testicular cancer having a common origin[57] is unknown.

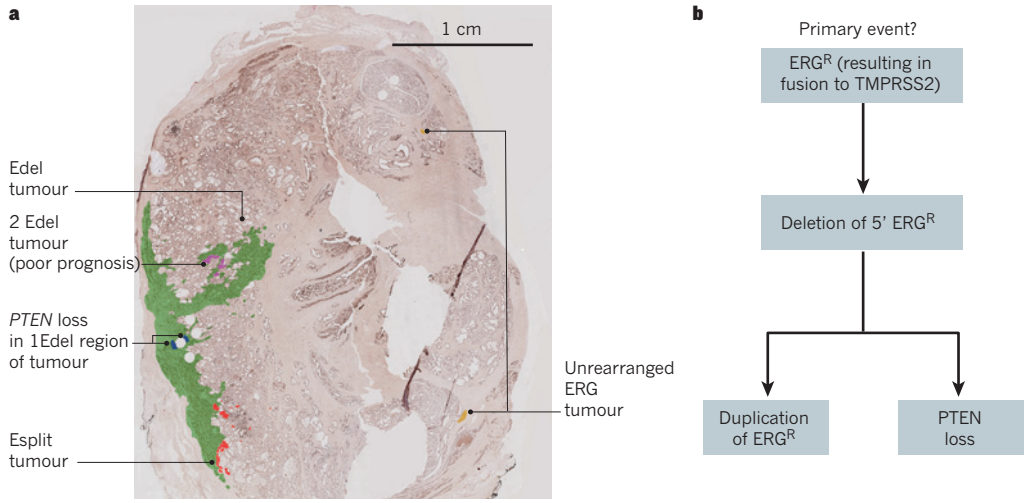

Figure 4 | Topography of cancer subclones. a, Tissue section of prostate to detect genetic events: *TMPRSS2–ERG* fusion (*ERG* via rearrangement (ERG^R)) and *PTEN* loss. **b**, The presumed sequence of clonal events.

the progression of cancer, or its resurgence after therapy, the primary unit of selection is the cell. This cell has to have extensive replicative potential, the so-called cancer stem cell (also known as the cancer-initiating or propagating cell) (Fig. 5).

The cancer stem-cell hypothesis was developed through transplantation experiments with leukaemic cells[66], and although it has been reported to be a general feature of all cancers[67], this idea is contentious. There has been no consensus on whether cancer stem cells are rare or high-frequency cells, or whether they have fixed, hierarchical or variable phenotypes, but considering the evolutionary progression in cancer, cells with extensive propagating activity are unlikely to be fixed entities[68,69]. Cancer stem cells are the cellular drivers of subclonal expansion and so probably vary in frequency and phenotypic features. The only feature they must have is the potential for extensive self-renewal (Fig. 5). Quantitative measures of stem-cell activity or self-renewal (through xenotransplantation or gene-expression signatures) can be used to predict the clinical outcome of several cancer types[70]. The cancer stem cell's ability to self-renew is made stronger by an aberrant genotype and, possibly, other, epigenetic, features. Several testable predictions can be made from this. First, cancer stem cells should evolve and change in genotype and phenotype as the cancer evolves before and after therapy. Some therapies may even provide a strong selection for cancer stem-cell survival and proliferation[71]. Second, as cancers progress, there should be selective pressure for the cells with the most extensive self-renewing capacity, but at the expense of cells with the ability to differentiate. This has been observed in chronic myeloid leukaemia (CML)[72] and mouse models[73,74]. A higher probability of symmetrical self-renewing proliferative cycles would be expected to result in an increased number and frequency of cancer stem cells. It is therefore of some consequence that loss of the *TP53* DNA damage checkpoint, which frequently correlates with cancer progression and clinical intransigence[75], seems to 'release' stem-cell-like transcriptional signatures[76] and leads to enhanced self-renewal in mammosphere culture systems[77]. The frequency of cancer stem cells could then increase from low to very high frequency as the disease progresses[78,79]. Third, for selection to operate through microenvironmental or therapeutic pressures, there should be contemporaneous genetic variation in cancer stem cells, which has been shown in leukaemias[33,80].

These considerations have significant clinical implications. Whatever the frequency and phenotype, if self-renewing cancer stem cells drive and sustain cancer-clone evolution, this suggests they are the repository of functionally relevant mutational events that drive clonal selection before and after therapy. This supports the view that cancer stem-cell restraint or elimination should be the aim of any therapy. However, if cancer stem cells are as genetically (and epigenetically) diverse as evolutionary considerations and initial experiments[33,80,81] indicate, this could be the reason for therapeutic failure. The adaptability of cancer stem cells provided by genetic diversity is added to by what seems to be their intrinsically lowered susceptibility to drugs and irradiation[82]. This may be because of the association with stromal cells[83] and the quiescence of cancer stem-cell subpopulations, as well as the properties of enhanced DNA repair and elevated expression of drug efflux pumps, which may be the evolved contingencies to protect normal stem cells.

Subclonal genetic heterogeneity is a common, if not universal, feature of cancers[84]. However, it cannot be assumed that all subclones are sustained by cancer stem cells; some could be evolutionary dead-ends generated by cells with only limited propagating potential. It is partly to accommodate this that the *in vivo* assay for cancer stem cells involves sequential transplants[66]. Ideally, the genomes of single cancer stem cells would be interrogated to investigate how they relate to subclones, but this is not currently possible. However, the genetic heterogeneity of cancer stem cells can be inferred by comparing subclonal diversity or clonal architecture before and after transplantation. Quadrant sections of glioblastoma have been shown to have divergent but related genotypes, but all sections contained cells that read-out in the *in vivo* (intracerebral) cancer stem-cell assay[85]. More definitive data come from comparing pre- and post-transplant subclonal genetic profiles that were investigated at the single cell level or by single nucleotide polymorphism arrays in B-cell precursor acute lymphoblastic leukaemia. Multiple subclones from each patient's diagnostic sample registered in the *in vivo* cancer stem-cell transplant assays, albeit with variable competitive potency[33,80,81]. We are still awaiting experimental confirmation that genetic diversity of cancer stem cells is a common feature of cancer, but, assuming that it is, this will have important therapeutic implications.

A Darwinian bypass

Nowell[3] stated in his landmark article "more research should be directed towards understanding and controlling the evolutionary process in tumours before it reaches the late stage seen in clinical cancer". Although cancer therapy has had its successes, in reality very few advanced or metastatic malignancies can be effectively controlled or eradicated. Genetic variation in cancer stem cells, particularly if induced by genetic instability, provides the opportunity for cells to escape and the therapy to fail. Other, non-genetic, mechanisms of positive selection by therapy exist, including signalling plasticity (or oncogene bypass)[86], quiescence[87] and epigenetic changes[88]; however, many of these depend on heritable, and thus selectable, epigenetic variation. Great expectation has been placed on the audit of cancer genomes that, by identifying recurrent and "druggable" mutations, would herald a new phase of highly specific or targeted

small-molecule inhibitors and personalized medicine[89]. Oncogene addiction may be the Achilles heel of cancer in this respect[90]. The success of imatinib and the derivative non-receptor tyrosine (ABL1) kinase inhibitors in CML[90] was very encouraging, but CML is not a typical cancer. It is essentially a premalignant (albeit ultimately lethal) condition, probably driven by a single founder mutation (*BCR–ABL1* fusion), which provides a universal target for therapy. Even in the most favourable of circumstances, escape occurs either by quiescence (and coupled resistance) of cancer stem cells[91] or by mutation of the ABL1 kinase target. Once CML has evolved to an overt malignancy or blast crisis, with increased genetic complexity, ABL1 kinase-directed therapy is often ineffective.

Other small-molecule inhibitors directed at mutant products have produced encouraging results in patients with advanced disease, but the benefits are transitory and cancer clones re-emerge with resistant features. When the targets selected are non-founder mutations, even if they are dominant in the neoplasm, therapy can be predicted to select for subclones lacking the mutant target[70]. Alternatively, subclones can have additional mutations that allow a bypass of the signalling pathway of the drug target, such as the MET proto-oncogene (*MET*) amplification in *EGFR* mutant lung cancer treated with EGFR kinase inhibitors[92].

Supporters of targeted therapy and personalized medicine argue that a combination of drugs that target components of networked signalling and are tailored to the individual patient's cancer genome is the solution to this problem. In this regard, synthetic lethal strategies seem promising[93].

Self-renewing cancer cells are the ultimate target for therapy, so high-throughput screening for selective inhibitors is an encouraging development[71]. Ways to target the components of the self-renewing process itself (independent of specific mutant genotype) deserve exploration, especially if a distinction can be made from normal adult stem cells. In the case of CML, intrinsically resistant (and possibly quiescent) stem cells, have been targeted by combining selective kinase (ABL1) inhibitors with inhibitors of a histone deacetylase[94] or BCL6 (ref. 95). Ultimately, it may prove difficult to thwart the plasticity and adaptability of cancer cells (or cancer stem cells), which are an inherent evolutionary feature of advanced disease, and a 'Darwinian bypass' may be required, for which there are a number of possibilities. An implication of the evolutionary diversity of cancer is that prevention (smoking cessation, avoiding sunburn, prophylactic vaccines, and so on) makes a great deal of sense, as does early detection and intervention (that is, before genetic diversification and dissemination become extensive).

An alternative therapeutic strategy is to focus on the microenvironmental habitat using 'ecological therapy', which aims to change the essential habitat and dependency of the cancer cells[96]. For example, anti-angiogenesis can provide a potent restraint on cancer stem cells[97]. Other examples are the use of bisphosphonates to remodel bone in patients with prostate cancer, the use of aromatase inhibitors in patients with breast cancer, exploiting hypoxia, the use of inhibitors of inflammation or tumour-infiltrating macrophages, and blocking cancer stem-cell interactions with essential stromal or niche components[96,98].

Another alternative is to control the cancer, rather than eradicate it, thereby turning cancer into a chronic disease. Because the speed of evolution is proportional to the fitness differential between the cells, cytotoxic drugs are predicted to select rapidly for resistance[5]. It is thought they cause competitive release[99] by removing all of the competitors of resistant cells. In contrast, cytostatic drugs should delay progression and mortality longer than cytotoxic drugs because sensitive competitor cells remain in the tissue to occupy space and consume resources that would otherwise be used by the resistant clones. In addition, by suppressing cell division, cytostatic drugs also suppress the opportunities for new mutations. A study by Gatenby and colleagues[100] showed that by treating an aggressive

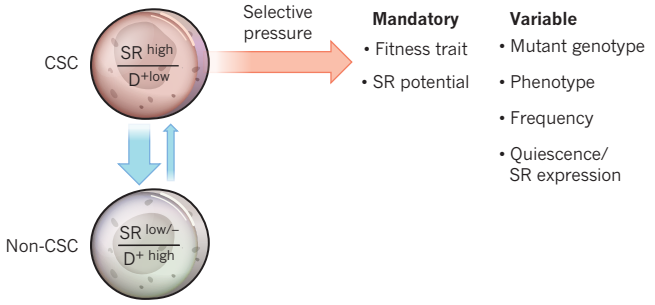

Figure 5 | Selective pressure on cancer stem cells. Selective pressures can include environmentally derived genotoxicity, natural or physiological restraints, cancer therapy, and so on. Mutation in progenitor cells can convert these cells back to a self-renewing population[72]. The small blue arrow represents a mutation; the large blue arrow represents differentiation: in both cases they represent a change in state. In addition to the mandatory trait of self-renewal, cancer stem cells (CSC), can exhibit any phenotypic feature that allows cells to continue to survive and proliferate in the face of a particular constraint. D^+, differentiation; SR, self-renewal.

ovarian cancer (OVCAR-3) xenograft tumour to maintain a stable size, rather than to eradicate it, host mice could be kept alive much longer. Moreover, the dose of carboplatin necessary to keep the tumour at a manageable size declined over time[100]. Researchers should now focus on what phenotypes can be selected for to make neoplasms less deadly and more clinically manageable.

The evolutionary theory of cancer has survived 35 years of empirical observation and testing, so today it could be considered a bona fide scientific theory. The basic components of somatic evolution are well understood, but the dynamics of somatic evolution remain unclear. Fortunately, there are evolutionary biology tools that may be applied to neoplasms to address many of the fundamental cancer biology questions, such as the order of events in progression, distinguishing driver from passenger mutations, and understanding and preventing therapeutic resistance. The dynamics of clonal diversification and selection are critical to understanding these issues. The challenge now is to use the clinical opportunities to address directly the evolutionary adaptability of neoplasms and design interventions to slow, direct or control cancer-cell evolution to delay or prevent mortality. ■

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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