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The causes and consequences of genetic heterogeneity in cancer evolution

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Recent studies have revealed extensive genetic diversity both between and within tumours. This heterogeneity affects key cancer pathways, driving phenotypic variation, and poses a significant challenge to personalized cancer medicine. A major cause of genetic heterogeneity in cancer is genomic instability. This instability leads to an increased mutation rate and can shape the evolution of the cancer genome through a plethora of mechanisms. By understanding these mechanisms we can gain insight into the common pathways of tumour evolution that could support the development of future therapeutic strategies.

Extensive genetic and phenotypic variation exist not only between tumours (intertumour heterogeneity) but also within individual tumours (intratumour heterogeneity; Fig. 1). In tumours, populations of genetically distinct subclones can intermingle or be spatially separated, and this subclonal architecture varies dynamically throughout the disease course[1,2]. Inter- and intratumour heterogeneity have significant implications for the choice of biomarkers to guide clinical decision-making in cancer medicine (see Siu and colleagues on page 355).

Genomic instability is a prominent source of genetic diversity within tumours, generating a diverse cell population that can be subject to selection in a given micro-environmental (see Review by Junttila and de Sauvage on page 346) or therapeutic context[3–5]. Genomic instability can arise through various routes, leaving distinct genomic footprints and differentially affecting tumour evolution and patient outcome. Different instability mechanisms can operate over the course of tumour development, and may be influenced by exposure to cancer drugs[6,7]. However, genomic instability might also be an attractive therapeutic target[8].

In this Review we summarize the evidence for genetic and phenotypic variation both within and between tumours, and discuss how this might affect tumour biology, drug response and patient outcome. We focus on the role of genomic instability in generating inter- and intratumour heterogeneity, and review the array of instability mechanisms observed in cancer and their contribution to tumour evolution.

Heterogeneity between different tumour types

It has long been appreciated that there are both genetic and functional differences between tumours. Tumours that originate from different tissues and cell types vary in terms of their genomic landscapes, prognosis and their response to cytotoxic therapies, probably resulting from the fact that the genetic events of transformation interact with cell-intrinsic biological properties. Reflecting this, the site of origin of the primary tumour frequently determines treatment decisions. However, considerable variation in terms of genomic aberrations, aggressiveness and drug sensitivity is also observed between tumours that originate from the same tissue and cell type[9–11].

Mutational frequencies of oncogenes and tumour suppressors vary between tumours of different tissues, probably reflecting the importance of distinct signalling pathways within specific tissues or cellular contexts. Mutations in the tumour suppressor gene *APC*, for instance, are common in gastrointestinal malignances such as colorectal cancer, probably owing to the importance of WNT-signalling pathways in intestinal epithelial homeostasis[12]. The specific cell type of origin can also profoundly influence tumour genetics and biology; for example, endocrine and exocrine pancreatic tumours exhibit considerable divergence in their mutational spectra and clinical behaviour[13]. Intrinsic differences between cell types may affect therapeutic response; the ability of cancer cells to undergo apoptosis in response to DNA-damaging agents seems to be substantially influenced by the cell type of origin[14]. Furthermore, drugs that target the mutant BRAF(V600E) oncoprotein seem to be less efficient in colorectal cancer cells than in melanoma cells[15]. This could be attributable to feedback upregulation of epidermal growth factor receptor (EGFR) after BRAF(V600E) inhibition in epithelial colorectal cancer cells but not in melanoma cells, which are derived from the neural crest and have lower basal EGFR expression[15].

The clinical challenge that intertumour variation presents can be met to some extent by classifying tumours into subgroups. These may predict, for example, patient outcome or drug sensitivity on the basis of mutations, copy number changes, protein or RNA expression profiles, or patterns of genomic instability[10,16,17]. For example, breast cancers are routinely classified according to expression of hormone receptors as well as amplification of the gene *HER2* (also known as *ERBB2*) and expression of its protein[10]. Similarly, the discovery that around 10% of lung cancers harbour *EGFR*-activating mutations has led to the use of small molecule EGFR inhibitors in the treatment of this disease[18].

However, recent advances in next-generation sequencing have revealed greater than expected inter- and intratumour genetic heterogeneity — very few mutations were observed in more than 5–10% of tumours of a particular tissue type[9]. In addition, within a cohort of tumours, the same gene may be affected by point mutation, DNA methylation, copy number alteration, or a combination of the three, underscoring the need for integrative approaches to analysing somatic aberrations in the cancer genome. Efforts to define tumour subgroups based on specific mutations may be confounded by epistasis, which involves the action of one gene on another. For example, in acute myeloid leukaemia (AML), *NPM1* mutations confer a favourable prognosis only in the presence of a co-occurring *IDH1* or *IDH2* mutation[19].

Although genetic heterogeneity between tumours is known to be widespread, it has long been appreciated that phenotypic heterogeneity

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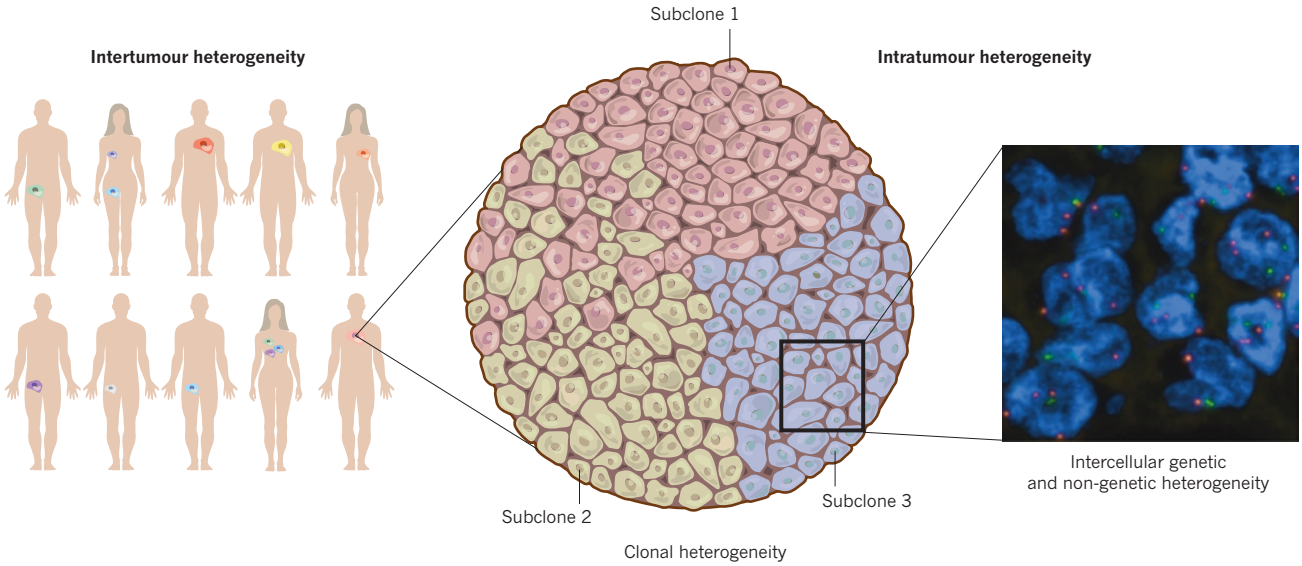

Figure 1 | Intertumour and intratumour heterogeneity. Genetic and phenotypic variation are observed between tumours of different tissue and cell types, as well as between individuals with the same tumour type (intertumour heterogeneity). Within a tumour, subclonal diversity may be observed (intratumour heterogeneity). Subclones may intermingle (as shown by subclones 1 and 2) or be spatially separated (as shown by subclone 3). Separation between subclones could reflect physical barriers such as blood vessels or micro-environmental changes. Tumour subclones may show differential gene expression due to both genetic and epigenetic heterogeneity. Within a subclonal population of tumour cells — shown here as a tumour section, hybridized to two fluorescent probes for the centromeres of two chromosomes (chromosome 2, red; chromosome 18, green) with DNA (blue) — there is intercellular genetic and non-genetic variation of, for example, chromosome copy number, somatic point mutations or epigenetic modifications that results in phenotypic diversity. Intercellular genetic heterogeneity is exacerbated by genomic instability, and may foster the emergence of tumour subclones. Genomic instability and tumour subclonal architecture may vary further over time if influenced by, for example, cancer treatment.

may not be as extensive, with the same cellular pathways often repeatedly affected[9,20–24]. Nevertheless, the impact of a given mutation will depend on the position and relative importance of the affected protein within the signalling pathway. An emerging theme from recent studies is that histone-modifying genes are recurrently mutated[1,9,22,25], suggesting that altered chromatin regulation could be a common phenotype in a range of tumours. Another unifying feature of many genetically diverse malignancies is genomic instability — the same pattern of instability can occur through various distinct routes. For instance, deregulation of the G1–S transition, which is observed in almost all cancers, can be achieved through multiple means, simultaneously overriding cellular senescence, promoting proliferation and destabilizing the genome through DNA replication stress[26–28].

Despite the evidence for functional convergence, it will nevertheless be challenging to identify biomarkers to define phenotypically similar, yet genetically diverse, lesions to guide treatment. In addition, the task of identifying and validating biomarkers may be complicated further by intratumour heterogeneity (See Review by Siu and colleagues on page 355).

Intratumour heterogeneity and tumour evolution

Within tumours, diversity occurs in the expression of protein biomarkers[10], and at multiple and different genetic[29,30] and epigenetic[30,31] levels. This diversity might complicate our ability to resolve the full spectrum of cancer pathway aberrations and confound biomarker validation through tumour sampling bias.

Genetically distinct subclonal populations of cells arise through intercellular genetic variation, followed by selective outgrowth of clones that have a phenotypic advantage within a given tumour micro-environmental context[32–34]. Fluctuations in subclonal architecture can occur, for example, in the context of disease progression (such as metastasis, see Review by Klein on page 365) or drug treatment[1,6,21,35–37]. A clonal sweep, whereby a new clone takes over the entire population, replacing ancestral clones, will result in a homogeneous cell population. During linear evolution, if a new clone fails to outcompete its predecessor, or predecessors, a degree of heterogeneity will be observed[38]. Branched tumour evolution, in which distinct subclones evolve in parallel, however, will result in extensive subclonal diversity[3].

A tumour's subclonal architecture can be reconstructed from sequencing approaches[22,36,39–41] or multiplex fluorescence *in situ* hybridization (FISH)[2], providing insight into cancer evolution. Branched evolution has now been documented in a range of tumour types, including adenoma-to-carcinoma transition of the colon[42], childhood acute lymphoblastic leukaemia (ALL)[2], chronic lymphoblastic leukaemia (CLL)[1], pancreatic cancer[35] and breast cancer[39,41]. In cases of ALL that exhibit branched evolutionary trajectories, heterogeneity has been observed between leukaemia-propagating cells[2]. This has important implications for cancer stem-cell models because it suggests that each subclonal branch was probably sustained by genetically distinct leukaemia-propagating cells[2]. It is conceivable that genetically heterogeneous subclonal lineages in solid tumours could also be maintained by distinct stem cells (See Review by Meacham and Morrison on page 328).

Branched evolutionary trajectories with spatial separation of tumour subclones (Fig. 1) have also been described. In clear-cell renal cell carcinoma, sequencing multiple biopsies from the same primary tumour revealed spatially separated subclones, harbouring heterogeneous somatic mutations and copy-number events[40]. Likewise, multiregion sampling in glioblastoma documented heterogeneous copy number events between different regions of the same tumour[43]. Evidence for clonal diversity between primary and metastatic sites has also been demonstrated in breast cancer[36], pancreatic cancer[21,35] and medulloblastoma[44]. Intratumour heterogeneity of spatially separated subclones, at the DNA copy-number level, may be so profound that the copy number profiles of single tumour biopsies may more closely resemble those of tumours from different patients than those of adjacent biopsies of the same tumour[45].

At metastatic sites, the new micro-environment, together with the selection pressures imposed by the process of metastasis itself, offer a plausible explanation for genetic differences between metastatic and primary sites[46] (see Perspective by Klein on page 365). However, it is

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BOX 1

Main pathways implicated in genomic instability

- **Base and nucleotide excision repair.** These pathways function to excise and repair abnormal bases or nucleotides, such as deaminated cytosines or ultraviolet radiation induced pyrimidine dimers. Germline mutations in components of these pathways predispose people to colonic polyposis[64] or skin cancers[66].
- **Mismatch repair.** This process acts during DNA replication to correct base mismatches, as well as insertions and deletions at repetitive sequences (microsatellites)[68]. Loss of function of *MSH2* and *MLH1*, which are required for mismatch repair, results in hypermutation and microsatellite instability. Mutations abrogating the proofreading ability of the DNA polymerases δ and ε increase base mismatches without affecting microsatellite stability[12,67].
- **Telomere maintenance.** Telomere erosion or uncapping results in catastrophic chromosomal instability through end-on chromosome fusions, followed by bridging during mitosis and further breakage, in 'breakage–fusion–bridge' cycles. Reactivation of telomerase expression in cancer cells can alleviate this instability[95].
- **Double-strand break repair.** Homologous recombination repair of double-strand breaks (DSBs) uses the sister DNA molecule as a template to repair the break, and is important for repair of stalled or collapsed replication forks. Non-homologous end joining directly ligates the DSB in an error-prone manner. Defects in DSB detection or repair result in chromosomal instability[8,81], with an elevated rate of structural chromosomal rearrangements[124] and point mutations[70].
- **DNA replication.** Deregulated DNA replication can result in replication fork stalling, reversal and collapse. Deregulation can occur through oncogene activation, loss of certain tumour suppressors, DNA polymerase inhibition, nucleoside imbalances, replication-blocking DNA lesions and clashes of replication forks with ongoing transcription. Such replication stress can trigger DNA DSB formation (particularly at genomic fragile sites), unscheduled recombination events and chromosomal rearrangements[26–28,87,97,125].
- **Chromosome segregation.** Defects in chromosome segregation can arise directly through defects in the mitotic checkpoint[73,74], sister chromatid cohesion[118], spindle geometry[75,78,79] and spindle dynamics[76,77]. These defects result in aberrant chromosome-spindle attachments, and missegregation of chromatids during anaphase, generating aneuploid daughter cells. Indirect segregation defects can be caused by structural chromosome rearrangements generated before mitosis, via replication stress[87,89], defective repair[90] or telomere fusion[80]. Further damage to missegregating chromosomes can occur during cytokinesis[85] or aberrant replication and fragmentation of micronuclei[86].

unclear precisely how spatial separation of genetically distinct clones arises in primary tumours[40]. It is possible that the separation reflects the presence of distinct micro-environmental niches in the primary tumour (see Junttila and de Sauvage on page 346), such that the subclones occupying each niche evolve relatively independently of one another. The similarity between this mode of tumour evolution and allopatric speciation (whereby geographically isolated populations from the same ancestral species evolve independently) raises the possibility that physical barriers in the tumour — such as blood vessels or tissue planes — prevent the mixing of tumour subclones (Fig. 1).

Nevertheless, intermingled heterogeneous clones are observed within single biopsies[22,39,41,47] to the extent that neighbouring cells can display amplification of different receptor tyrosine kinases — proteins that regulate key signalling processes and whose dysregulation has a crucial role in tumorigenesis[48,49]. At this intercellular level, genomic instability is thought to play a crucial part in generating enhanced genetic diversity. This intercellular heterogeneity increases phenotypic variation, broadening the pool of cells that are subject to selection, and therefore the likelihood of selective expansion of multiple different subclones and the emergence of a complex subclonal tumour architecture[4].

Genomic instability and tumour evolution

The genome is replicated and divided with high fidelity, such that the endogenous point-mutation rate in somatic cells is relatively low (0.77×10^{-9} per site per cell division[50]) and errors in chromosome segregation are both rare (roughly 1 per 100 cell divisions) and poorly tolerated in non-transformed proliferating cells[51–53]. Disruption of mechanisms that maintain genome integrity or exposure to exogenous mutagens will elevate the mutation rate (mutation here is used in the broadest sense to include any genome aberration). Most solid tumours and haematopoietic malignancies display at least one form of genomic instability[54].

Despite the clear association between genomic instability and cancer, there has been considerable debate as to whether elevated mutation rates are necessary for tumorigenesis[55,56]. Even with a normal somatic point mutation rate, the diversity and mutational burden within a large tumour cell population is likely to be extensive[55], and sequencing studies in AML and colorectal cancer have found that there is no need to invoke elevated mutation rates to explain the number of mutations observed[20,38]. Nevertheless, although not strictly necessary for carcinogenesis, the observation that most tumours display some form of instability suggests that it provides some benefit to tumour evolution. This hypothesis is supported by mouse models, which have revealed that genomic instability increases the risk of carcinogenesis[57,58], leads to tumour regrowth after oncogene withdrawal[59] and promotes loss of heterozygosity of tumour suppressor genes[60].

Different genomic instabilities result in different genetic lesions, ranging from increased point mutation frequency to small insertions and deletions, large-scale chromosomal rearrangements and alterations in ploidy (see Box 1 and Table 1). However, the phenotypic penetrance of these genetic changes may vary; a chromosome gain or loss is more likely to have functional consequences than a point mutation, most of which are neutral. As different patterns of genomic instability have distinct genomic footprints, it is possible to interrogate sequencing and copy-number data to examine how genomic instability has shaped tumour growth and evolution[61–63]. Given that most studies rely on examining a pooled population of cells, the true level of intratumour genetic heterogeneity driven by genomic instability is likely to be much greater than estimated, owing to both sampling bias and the inability to resolve heterogeneity between single cells. A recent study analysed an extensive catalogue of mutations in 30 of the most common cancer types and uncovered more than 20 signatures of processes that mutate DNA, driven by both exogenous and endogenous factors[63].

In addition to global effects on genome architecture and mutational patterns, specific mutations may occur at an increased rate in the context of a particular instability mechanism. This is illustrated in colorectal tumours, in which those with defective mismatch repair exhibit a strikingly different mutational profile from those tumours with intact repair[12]. Similarly, colorectal tumours with defective base excision repair, due to *MYH* (also known as *MUTYH*) mutations (Box 1) show a preponderance of C·G→A·T transversions in *APC*[64]. In lung cancer, smoking increases the mutation rate and the frequency of C·G→A·T transversions[22] such that mutations in *TP53* can often be directly attributed to DNA damage from cigarette smoke carcinogens[65]. This effect

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is likely to be compounded by epistasis, whereby a given mutation that occurs more frequently in the context of one pattern of instability may then lead to selection for specific combinations of mutations.

Mechanisms of genomic instability

Increased point mutation frequencies can be caused by both exogenous factors[22,63] and defects in DNA repair pathways, such as mismatch repair and base and nucleotide excision repair[12,64,66–68] (see Box 1 and Table 1). In addition to hypermutation, mismatch repair deficiency is associated with expansion and contraction of repetitive tracts of DNA (microsatellite instability), leading to an increased frequency of frameshift mutations[68]. Elevated point mutation frequencies may also arise through mechanisms that affect gross chromosome structure, such as DNA replication stress[69] and defects in homologous recombination[70].

Different mechanisms of instability can lead to particular distributions of point mutations — replication stress is associated with an enrichment of mutations in large genes[69], whereas microsatellite instability may affect specific genes more frequently than others owing to the nucleotide composition of those genes[68]. Highly localized clusters of mutations that are associated with chromosomal rearrangement, known as kataegis, may be caused by the action of the APOBEC family of cytidine deaminases in breast cancer[61]. A recent study has highlighted the need to consider variation in mutation rate across the genome when identifying putative driver mutations; mutation frequency across the genome was found to strongly correlate with DNA replication timing and transcriptional activity[11].

Chromosomal instability occurs at high frequencies across many cancer types and is associated with aggressive disease, drug resistance and poor prognosis[54,71]. This form of instability refers to an increased rate of change in chromosome number or structure, and results in a range of karyotypic abnormalities, including whole-chromosome and segmental aneuploidies, as well as translocations, inversions and deletions (Table 1). A substantial proportion of chromosomally unstable tumours also show increased ploidy, leading to suggestions that genome doubling could act as a precursor to chromosomal instability[72].

Chromosomal instability can arise through a range of both mitotic and pre-mitotic defects. Mitotic defects result in inaccurate partitioning of whole chromosomes between daughter cells — mediated through incorrect attachment of chromosomes to the mitotic spindle. This may occur through inadequate detection and correction[73,74], or through increased formation, of incorrect attachments as a result of altered microtubule dynamics or transiently abnormal spindle geometry[75–79]. Pre-mitotic defects that converge on defective repair or increased generation of DNA damage can affect chromosome structural integrity. DNA damage may be acquired from exogenous sources (such as radiation), but in general chromosomal rearrangements in cancer cells are thought to arise through endogenous DNA damage. This may be, for example, through telomere dysfunction[80] or defects in DNA repair pathways (such as *BRCA1* and *BRCA2* mutations, which result in defective homologous recombination[8,81]; Box 1). A likely source of much of the DNA damage in tumours is DNA replication stress[26,27] (Box 1), which is reflected by the enrichment of chromosome breakpoints and loss of heterozygosity in cancer genomes at fragile sites (regions of the genome that are particularly susceptible to replication stress)[82–84].

In recent years, there has been a growing appreciation that, from a mechanistic perspective, numerical and structural instability cannot be considered in isolation[80,85–87]. Mitotic defects in chromosome attachment, typically thought to lead to whole-chromosome aneuploidies, have been shown to result in DNA damage and structural rearrangements, through entrapment of chromosomes in the cytokinesis furrow[85] or through aberrant replication and fragmentation of micronuclei[86]. Conversely, structural chromosome rearrangements can also compromise the accuracy of chromosome segregation during mitosis[80,87–90]. Therefore, the relative contributions of mitotic defects and pre-mitotically generated structural chromosome abnormalities to chromosomal instability in tumours need to be explored.

Variation in genomic instability over time

Tumour evolution is a dynamic process that contributes to both spatial and temporal tumour diversity. The type of genomic instability operating in a tumour at any point in time is likely to have a significant impact on tumour evolution, not only in terms of which specific pathways are dysfunctional[91], but also in terms of the likelihood of a tumour

Table 1 | Characteristic features of cancer genomes

Genomic feature	Description	Possible mechanisms
Whole-genome duplication	Duplication of the entire DNA complement	Cell fusions, cytokinesis failure, metaphase or anaphase defects, or endoreduplication[72]
Chromosomal loss and gain	Loss and gain of whole chromosomes and segments of chromosomes	Mitotic defects: abnormal spindle geometry[75,78,79], centriole amplification[75,78], mitotic checkpoint defects[73,74] or hyperstable microtubules[76,77] Pre-mitotic defects: cohesin mutations[118], DNA replication stress[27,28,82,87], DNA damage repair defects[81] or telomere dysfunction[80,95]
Chromothripsis	Extensive genomic rearrangements involving one or more chromosomes	Ionizing radiation, micronucleus fragmentation or replication defects[86,94,119]
Translocations	Chromosomal rearrangements resulting in fusion of two chromosome arms	Non-homologous end joining, aberrant lymphocyte DNA recombination mechanisms and cytokinesis-induced DNA damage[84,85,120]
Tandem duplications	Sequential copies of a genomic segment that often spans several genes	Unknown[121,122]
Loss of heterozygosity at fragile sites	Copy number imbalance or gene conversion at genomic loci that are particularly sensitive to replication stress	DNA replication stress[27,82]
Focal deletions	Deletion of less than 5 megabases	Chromosome breakage or DNA replication stress[82]
Focal amplification	Amplification less than 5 megabases	Double minute chromosomes generated through chromosome breakage[123]
Hypermutation	Elevated mutation rate, leading to abundance of somatic mutations	DNA mismatch repair defects[68], *BRCA2* mutations[70] and proofreading defects[67]
Kataegis	Localized hypermutation	AID/APOBEC activity[61]
C·G>T·A transitions	Base changes	Ultraviolet radiation-damage[63], alkylating agents[7] and spontaneous deamination of methylated cytosines[50]
C·G>A·T transversions	Base changes	Base excision repair defects[64], tobacco-related DNA damage[22] and cytotoxic chemotherapy[6]

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metastasizing[92] and its response to treatment[7,93].

Recent studies have revealed evidence for changing patterns of instability during tumour progression[31,39,40]. For example, in a study of clear-cell renal carcinoma, we found that chromosomally unstable metastases were probably derived from a tetraploid subclone in the primary tumour, whereas the remainder of the sampled regions in the primary tumour were diploid[40]. In a study of breast cancer, researchers made use of variant allele frequencies and copy number events that were derived from whole-genome sequencing reads to dissect the temporal sequence of mutations[39]. The mutations that occurred early in cancer development — on the trunk of the tumour's evolutionary tree — were found to exhibit a mutation pattern that was largely similar to germline mutations[50]. However, the mutations that occurred later during tumour progression had significantly altered mutational patterns, involving decreased frequencies of C·G→T·A base transitions[39]. These studies suggest that temporally separated biopsies may give strikingly different pictures of a tumour's genomic landscape.

Sequencing studies have also allowed researchers to analyse how tumour evolution and genomic instability could be affected by therapy. The analysis of pairs of primary and relapsed tumour cells from eight patients with AML found relapse-specific mutations and an increase in base transversions after chemotherapy, highlighting how treatment itself can act as a transient initiator of exogenous instability[6]. A study of nine glioblastomas from patients who had undergone chemotherapy with the alkylating agent temozolomide found that two cases exhibited an elevated frequency of mutations with a pattern that was consistent with induction by alkylating agents[7]. Both of these hypermutator gliomas relapsed after chemotherapy, and mutations in the mismatch repair gene *MSH6* (Box 1) were detected in the relapsed but not in pretreatment samples. This suggests that treatment with alkylating agents can impose selection for loss of mismatch repair function, allowing propagation of therapy-induced mutations. Because chromosomal instability is associated with multidrug resistance[93] and poor prognosis[71], this raises the possibility that this form of genome instability may also be selected for during treatment.

It is important to note that observations of high numbers of mutations or chromosomal rearrangements do not necessarily imply ongoing genomic instability, but may instead reflect a historical prolonged period of mutational acquisition, or alternatively the occurrence of transient mutational bursts[38,94]. The evolution of the cancer genome may proceed not only in a gradual manner through clonal selection of point mutations or chromosomal alterations, but may also be subject to punctuated phases characterized by drastic changes. It has been postulated that chromothripsis, in which tens to hundreds of chromosomal rearrangements occur across one or a few chromosomes during a single catastrophic event, represents a major evolutionary leap in terms of tumour development[94]. Similarly, telomere dysfunction catalysed by eroded chromosome ends may be transient, leading to catastrophic instability until telomerase expression is activated to stabilize chromosome ends[95] (Box 1). Nevertheless, although these punctuated events are likely to contribute to cancer-genome evolution, observations of an active DNA damage response, and variation in chromosome copy number in both early stage and advanced malignancies, suggest that ongoing instability occurs in a substantial proportion of tumours[27,96,97].

Drastic genomic change may also result from genome doubling, which has been estimated to occur at frequencies as high as 60% in some cancer types[98]. Some studies have proposed that the process is an intermediate stage on the path to aneuploidy, precipitating genetic and functional intratumour heterogeneity through chromosomal instability (see ref. 72). Although genome doubling has been observed as an early event in cancer development[99], recent sequencing studies have found evidence for it at later stages in disease progression[39]. Genome doubling has been observed after, rather than before, the onset of chromosomal instability, indicating that there may be additional evolutionary benefits to genome doubling beyond catalysing chromosome reassortment[39,98]. Furthermore, genome doublings are proposed to play a major part during the evolution of eukaryotes, creating evolutionary opportunities by providing new genetic material for mutation and selection to act upon[100]. In tumour evolution, it is possible that both genome doublings and tandem duplications (Table 1) might facilitate subfunctionalization of duplicated genes.

Intertumour variation in genomic instability

There is considerable heterogeneity in both the patterns and extent of genomic instability that are prevalent in different tumour types. Microsatellite instability is observed most frequently in colorectal tumours; however, it occurs only in a minority of these tumours[16]. This instability seems to preferentially occur in tumours of the proximal colon, suggesting an association with epigenetic or environmental factors[68]. By contrast, defective homologous recombination is observed more frequently in breast and ovarian cancers[8]. A given pattern of instability can be caused by multiple mechanisms; for example, chromosomal instability may be driven by different defects in different tumours[52,75,87]. Furthermore, based on cell-line studies, multiple defects can operate simultaneously in a given tumour cell[52,75,78,87].

Different instability mechanisms are also associated with variations in clinical outcome, for example, colorectal tumours with microsatellite instability are associated with a better patient prognosis compared with chromosomally unstable tumours[17]. Differences in disease outcome could relate to the profound infiltration of immune cells observed in microsatellite unstable tumours compared with colorectal cancers that display chromosomal instability. This immune infiltrate may be driven by an increased repertoire of neoantigens in these tumours as a result of mismatch repair deficiency[101]. Alternatively, the hypermutator phenotype of microsatellite-instability tumours could place them at the limit of tolerable instability and treatment with mutagenic chemotherapy may push them over the threshold[4]. Consistent with this idea, *in vitro*, microsatellite-instability colorectal cancer cell lines are more sensitive than chromosomally unstable cell lines to a range of different targeted and cytotoxic agents[93]. Whatever the explanation, these observations suggest that not all forms of genetic heterogeneity are equal in terms of their impact on patient outcome.

In addition to variation in the pattern of instability between tumours, variation also occurs in the degree of instability between tumours. For example, in breast cancer, oestrogen receptor positive (ER^+) tumours display significantly lower karyotypic complexity (a genomic footprint of chromosomal instability) , compared to ER^- tumours[96]. This variation in the degree of chromosomal instability is associated with different outcomes; we have found evidence in ER^- breast cancer, squamous non-small-cell lung cancer, and serous ovarian and gastric cancer that tumours within the highest quartile of chromosomal instability have a better prognosis than those in intermediate quartiles of chromosomal instability[96,102]. These data suggest that the benefits of genomic instability must be balanced against its costs, although it is unclear whether the link with prognosis is independent of treatment or whether therapy itself can push the level of instability beyond the tolerable threshold.

An elevated mutation rate not only increases the supply of potentially beneficial mutations but also deleterious ones, and there may be an optimal level of instability, which if exceeded impedes tumour growth, a scenario analogous to mutational meltdown in bacteria or error catastrophe in viruses[4]. Examples of excessive instability resulting in cell lethality can be observed in the context of mitotic checkpoint dysfunction[57,103] or multipolar anaphases in cells with extra centrosomes[75]. Cells may adapt to limit catastrophic instability by clustering extra centrosomes to form a bipolar spindle and avoid lethal multipolar division[75,78], and so efforts to prevent centrosome clustering for therapeutic purposes to exacerbate instability may be effective[54]. Similarly, excessive instability in the context of *BRCA1* and *BRCA2* mutations may be averted by downregulation of the p53-binding protein 53BP1, to rescue, in part, homologous recombination repair[104]. By understanding cellular processes and pathways such as these — which are required for tolerance or averting excessive levels of genomic instability

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— therapeutic opportunities may be revealed. This is exemplified by the efficacy of poly(ADP-ribose) polymerase inhibitors in breast and ovarian cancers that bear *BRCA1* and *BRCA2* mutations[8,105,106]. Other possible means of targeting genomic instability include inhibiting replication checkpoints in cells undergoing replication stress[107] and perturbing pathways that are essential for the survival of aneuploid cells[51,53,93]. Further research is required to identify new strategies to target genomic instability in cancer, which may represent an attractive approach to treating heterogeneous tumours.

Consequences of intratumour heterogeneity

Similar to that observed between different tumours, some degree of phenotypic convergence may also occur between genetically distinct tumour subclones[40]. However, intratumour genetic heterogeneity also results in phenotypic diversity, and this has been documented in a range of tumour types, affecting clinically relevant parameters such as gene-expression signatures that reflect prognosis, and response to therapeutic agents[6,40,48,108]. In renal cancer, distinct transcriptomic profiles that reflect both good and poor prognosis have been described within one primary tumour[40], and sampling of 11 glioblastomas revealed that many patients displayed multiple transcriptomic subtypes within different biopsies from the same tumour[43].

Increasing evidence suggests that efforts to forecast outcome require the identification of low-frequency genetically and functionally distinct subclones at diagnosis[1,37]. This is illustrated by a recent study in chronic lymphocytic leukaemia, which revealed the selection of subclones that harbour distinct driver events during therapy[1]. The eight patients that harboured a detectable subclonal driver in their pretreatment samples showed significantly shorter time to re-treatment or death compared with the four patients with no evidence of subclonal driver events. Similarly, in a case of multiple myeloma, a subclone that was barely detectable at diagnosis was shown to be dominant towards the end of the disease[37]. These data underscore the need for longitudinal tumour sampling over the disease course and throughout treatment because the subclone that influences outcome may not be detectable in a single biopsy, or indeed even in multiple biopsies, taken before treatment.

Subclonal driver mutations result directly in phenotypic variation, for example in alterations to signal transduction pathways. In clear-cell renal cell cancer, a heterogeneous *MTOR* mutation (resulting in constitutive mTOR kinase activity *in vitro*) was associated with differential mTOR signalling pathway activation after 6 weeks of mTOR inhibitor therapy[40]. In a heterogeneous glioblastoma multiforme cell population harbouring mosaic amplification of *EGFR* and *PDGFRA*, combined inhibition of both kinases was necessary to attenuate PI(3)K pathway activity *in vitro*[48]. Intermingling of tumour cells harbouring amplification of distinct receptor tyrosine kinases (cells harbouring amplification of both genes were rare) hints at a possible functional codependency between the two genetically distinct populations[48,49], as has been demonstrated in models using the fruitfly *Drosophila melanogaster*[109]. Intriguingly, *EGFR* mutant tissue, cell lines and stem cells release interleukin 6 (IL-6) and leukaemia inhibitory factor, which contribute to enhanced growth of adjacent subclones through activation of wild-type EGFR[108], providing further evidence of possible codependencies between minor and major subclones in tumours.

However, it is important to note that phenotypic heterogeneity is not only mediated through genetic diversity. Genetically homogeneous subclones can behave in functionally distinct ways after exposure to chemotherapy, mediated by varied patterns of proliferation, with dormant resting cells surviving cytotoxic exposure[110]. Phenotypic heterogeneity is not determined solely through genetic distinctions between subclones, but also through stochastic events in gene expression and protein stability, epigenetic divergence and micro-environmental fluctuations[30,31,110,111]. Indeed, a study examining the fate of daughter cells derived from the same mother cell after antimitotic drug exposure found that the fates of the sister cells seemed to be independent of each other[112].

Intratumour heterogeneity and treatment strategies

Observations of intratumour heterogeneity pose a challenge to targeted therapies and raise important questions regarding future drug-development strategies, which may benefit from considering whether clonally dominant events may be more effective targets than subclonal lesions[29]. Founder events might include *EGFR* and *KRAS* mutations in lung cancer, VHL loss-of-function through mutation, methylation or homozygous copy number loss in clear-cell renal cell carcinoma[40], and *KRAS* and *BRAF* mutations in colorectal carcinoma[113]. Of these examples, only *EGFR* and *BRAF* mutations are currently directly targetable.

In addition, targeting clonally dominant genetic events is complicated by observations that a founder event in one tumour can be a later event in others. For example, in lung cancer, activating *EGFR* mutations were found to display intratumour heterogeneity and spatial discordance in 6.3–28% of cases[114], suggesting that in these tumours *EGFR* mutation was not a founder event. Even in tumours in which *EGFR* mutations are founder events, the presence of low-frequency subclones harbouring the T790M gatekeeper mutation, which confers resistance to EGFR tyrosine kinase inhibitors, have been shown to be associated with early disease progression in patients treated with EGFR inhibitors[114]. In patients with colorectal cancer who have undergone anti-EGFR antibody therapy, mutations in *KRAS* (which confer drug resistance) were detectable in circulating tumour DNA 5–6 months after therapy in 9 out of 24 tumours, 3 of which developed multiple distinct *KRAS* mutations. Mathematical modelling suggested that low-frequency subclones harbouring these *KRAS* mutations were present in the tumour before treatment[115]. The same challenges are likely to affect the efficacy of other targeted therapies.

Future directions

As genomics technologies advance further and become more affordable, our understanding of how genomic instability shapes tumour evolution will be greatly enhanced. Both mathematical and experimental modelling approaches are also likely to inform our understanding of how heterogeneity affects tumour progression[5,110,115,116]. Longitudinal tumour sampling approaches will be essential to decipher the impact of tumour heterogeneity on cancer evolution, and developing minimally invasive methods to profile heterogeneous tumour genomes will play a major part in deciphering changes in the genomic architecture of tumours in real time, exemplified by circulating free tumour DNA analysis[115]. In addition, there is a need to develop clinically useful measures of heterogeneity[117]. The prevalence of different patterns of instability will be more robustly assessed in large patient cohorts, and new patterns will probably be identified as the wealth of genomic data is analysed[63]. It will be important to examine in greater depth the relationship between the rate of instability and the complexity of subclonal architectures, as well as whether certain forms of genomic instability are associated with a greater degree of intratumour heterogeneity. There is a crucial need to understand mechanisms driving genomic instability so that therapeutic approaches to limit cancer diversity, adaptation and drug resistance can be developed. ■

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