Published in final edited form as:
Nat Rev Genet. 2012 November ; 13(11): 795–806. doi:10.1038/nrg3317.

Evolution of the cancer genome

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Abstract

The advent of massively parallel sequencing technologies has allowed the characterization of cancer genomes at an unprecedented resolution. Investigation of the mutational landscape of tumours is providing new insights into cancer genome evolution, laying bare the interplay of somatic mutation, adaptation of clones to their environment and natural selection. These studies have demonstrated the extent of the heterogeneity of cancer genomes, have allowed inferences to be made about the forces that act on nascent cancer clones as they evolve and have shown insight into the mutational processes that generate genetic variation. Here we review our emerging understanding of the dynamic evolution of the cancer genome and of the implications for basic cancer biology and the development of antitumour therapy.

Cancer is a disease of the genome. The classic model of carcinogenesis describes multiple, successive clonal expansions driven by the accumulation of genomic changes or ‘mutations’ that are preferentially selected by the tumour environment[1,2,3]. This traditional picture of linear cancer genome evolution has become more nuanced over the past decade as the research scalpel allows ever-sharper prosection of the underlying biology (FIG. 1; BOX 1).

Recent advances in sequencing technologies have delivered, for the first time, the opportunity to scrutinize all expressed genes (‘transcriptomes’), all exons (‘exomes’) and whole cancer genomes at base-pair resolution[4]. A number of different sequencing platforms now exist, including pico-titre plate pyrosequencing and ligation-based sequencing. From the viewpoint of understanding cancer genome evolution, the key aspect of this generation of sequencing technologies is that billions of independent sequencing reads are generated in parallel, with each read deriving from a single molecule of DNA. Thus, albeit with some sampling biases, the data represent a random sample of DNA molecules (and hence the genomes of individual cells) contained in the tumour sample. By contrast, the data derived from conventional genomic approaches, such as capillary sequencing or copy number arrays, are aggregate signals from many thousands of DNA molecules (BOX 2). Harnessing the attractive statistical properties of massively parallel data thus enables us to draw robust inferences about the mutational mix of a tumour sample, generating unprecedented insights into the fundamental genomic events that underlie the development of cancers and the order, rate and mechanisms by which they occur[5–7].

These approaches have been used to generate comprehensive catalogues of somatic mutations by comparing the genomic sequence of DNA taken from a patient’s cancer cells to the sequence of their germline DNA[7,8]. In particular, these studies have given an

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Competing interests statement The authors declare no competing financial interests.

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indication of the heterogeneity in cancer genome evolution, across tumour types, across individuals within a given tumour type and even within a single individual's tumour[9,10]. In this Review, we provide an initial overview of recent strategic and methodological developments in cancer genomics. Heterogeneity is central to cancer genome evolution, and we describe this at the level of cancer genes and within individual patients. We consider the evidence for the role of different processes, gradual and abrupt, by which heterogeneity may arise. Finally, we present the evidence for an elevated mutation rate in shaping cancer evolution.

Recent strategic and methodological advances

Tumour multi-sampling strategies

With the objective of understanding how the cancer genome varies over space and time, various groups have carried out studies of tumours and their respective non-malignant tissues obtained from an individual patient. These approaches may be broadly divided into 'geographical' and 'longitudinal' sampling strategies. 'Geographical sampling' encompasses those studies that compare multiple samples from an individual cancer that have been obtained at a single point in time. These samples may be derived from geographically distinct areas within a single large tumour mass and/or may include metastatic deposits in lymph nodes or distant organs[11,12]. 'Longitudinal sampling', by contrast, compares samples obtained at different time points in the life history of a cancer: for example, at diagnosis, relapse and metastasis[7]. A limited number of published studies have included samples that are separated by both space and time[9]. The biological question posed and the clinical feasibility largely determine the sampling strategy.

Single-cell sequencing

Single-cell sequencing is a potentially useful approach towards the study of cancer evolution and is the ultimate resolution of the multi-sampling approach. In proof-of-principle studies, this approach has been successfully applied to generate catalogues of point mutations in protein-coding regions and copy number changes[10,13,14]. These approaches have a requirement for whole-genome amplification of the genome of each cell, and this introduces several biases, with the potential for both false-positive and false-negative mutation calls. For haematological malignancies, *in situ* hybridization techniques allow single cells to be studied for cytogenetic abnormalities[15], and it is feasible that in the future, microfluidic techniques will allow cells to be isolated and analysed in one step for solid tumour samples as well[16,17]. The ability to make inferences about phylogenetic structure using single-cell sequencing will, however, still be fundamentally limited by how representative the biopsy sample is of the whole-tumour bulk and by how many cells are individually analysed.

Mathematical algorithms

Mathematical models have been widely applied in an attempt to unpick the complex and multifactorial influences on cancer progression[18–20]. Massively parallel sequencing data are particularly amenable to mathematical analysis because they represent a random sample of DNA molecules, and hence of individual cancer cell genomes, within a tumour specimen (BOX 2). Statistical algorithms for exploiting these properties have been developed, providing important insights into the clonal mix of the sample sequenced. For example, using the fraction of reads reporting a point mutation, the copy number at that locus and the level of normal cell contamination, we can work out whether the mutation is likely to be clonal or subclonal and whether the mutation has been duplicated by a subsequent copy number change[7,21,22–24]. Within a given copy number segment, this mandates a clear temporal precedence. The earliest mutations are those that are subsequently duplicated, followed by those that are clonal but that are present on a single copy of the locus and then

by those that are subclonal. This allows inferences about the relative timing of the copy number gain and about the changing mutational signatures that are operative in the different epochs[22,25].

With the exception of more complex processes such as chromothripsis (discussed below), genomic rearrangements generally represent simple events (such as deletions or inversions), occurring over the evolutionary time course of a cancer. Mathematically, these rearrangements can be considered as sequential selections from a known library of genomic transformations — remarkably, the constraints imposed by the simplicity of the repertoire of possible rearrangement types, the genome-wide, allelespecific copy number data and the observed breakpoints mean that even deeply complex clusters of rearrangements can be disentangled to yield both the final genomic configuration of segments and the temporal order in which the rearrangements occurred[26].

Mutations occur in a given genomic context, and this can also be exploited to understand cancer evolution. In particular, mutations can be ‘phased’ with nearby heterozygous germline SNPs, allowing haplotypespecific analysis of clonal and subclonal mutations[24]. Furthermore, pairs of mutations can be phased relative to one another, allowing patterns of branching and subclonal evolution to be delineated[5,24] (BOX 2). Although such approaches are currently limited to samples with hypermutable regions or with a high mutation burden, the increasing read lengths coming in future generations of single-molecule sequencers will vastly expand the power of this approach.

The heterogeneous cancer genome

The cancer genome is characterized by heterogeneity that is seen across tumour types, among cases of a particular tumour type and even within an individual cancer. This heterogeneity reflects the action of the twin evolutionary forces of variation generation and selection. The extent of genomic variability is testament to the diverse and dynamic nature of these forces.

The heterogeneity of cancer genes

Massively parallel sequencing has enabled us to construct nearly comprehensive catalogues of every mutation within an individual cancer genome at a single point in time[6]. To date, using conventional and newer technologies, almost 500 cancer genes have been identified[27]. In a handful of cancer types, specific underlying cancer genes are consistently mutated, such as the oncogenic fusion protein *BCR–ABL* in chronic myeloid leukaemia (CML) or inactivating mutations in the tumour suppressor gene retinoblastoma 1 (*RB1*) in retinoblastomas[28]. Specific cancer genes have also been implicated in the development of the same rare cancer type in different tissues. The oncogenic fusion gene *MYB–NFIB*, for example, drives the development of adenoid cystic carcinomas that arise in both breast and salivary tissues[29].

These examples, however, remain the exception rather than the rule. Most common cancers are associated with many diverse cancer genes that are mutated at a low frequency. One of the most striking observations from large cancer databases is the genetic heterogeneity between cancers and even within individual cancer types. The Cancer Genome Atlas Project analysed 489 high-grade serous ovarian cancers, and among the thousands of somatic mutations identified, only 10 of these were recurrently mutated cancer genes, and all but *TP53* were present in less than 10% of cases. The recent genomic analysis of 77 oestrogen-receptor-positive breast cancers also identified that most recurrent mutations occur infrequently, but they do cluster within a limited number of cellular pathways that are central to tumour cell biology[30].

Long-standing evidence indicates that breast cancer exhibits heterogeneity in terms of clinical behaviour and response to therapy. More recently, the genomic diversity underlying this heterogeneity has been documented[25,30–32]. For example, identification of a *TP53* mutation in breast cancer correlated with a higher proliferation index before therapy and less dramatic suppression of proliferation during therapy with an aromatase inhibitor[30]. The conventional subclasses of breast cancers are based on histopathological type and grade, immunohistochemical analysis of hormone receptors and overexpression of human epidermal growth factor receptor 2 (HER2; also known as ERBB2). However, in the past few years, these categories have been extended by molecular profiling studies that use expression analysis to reclassify breast cancers with unique biological and prognostic features[33]. These categories, which can be identified on gene expression profiles, reflect to some extent the underlying genomic profiles of the tumours[31], and it will be interesting to see how integrative transcriptional and genomic studies define this further in the whole-genome sequencing era.

The heterogeneity of the mutational landscape

In addition to the heterogeneity of cancer genes, there is considerable diversity in the nature, number and distribution of mutations within and across different cancer histologies[25]. Recent studies have revealed, for example, that the childhood cancers retinoblastoma and medulloblastoma contain substantially fewer somatic substitutions than do common adult-onset solid tumours and haematological malignancies, such as breast cancer or acute myeloid leukaemia (AML)[7,34–37]. This extends even to specific subtypes of tumours — for example, the number of mutations among individual HER2-positive breast cancers differed by a factor of six in a recent study[25].

Patterns of structural variants differ across tumour types: breast and ovarian cancers show many more tandem duplications than other tumour types do[38,39]; pancreatic cancer is characterized by frequent breakage–fusion–bridge cycles of chromosomal rearrangement[12]; prostate cancer shows balanced chains of rearrangements[40,41]; and various cancers, especially sarcomas and neuroblastomas, demonstrate chromothripsis (discussed below)[37,42,43]. Similarly, patterns of base substitutions differ extensively across tumours, depending on DNA repair defects and carcinogenic exposures[8,44,45]. Many of the pathways underlying the acquisition of somatic mutations in these cancers are poorly understood. For example, at least six or seven distinct point mutational signatures can be identified in breast cancers, of which only one or two can currently be attributed to known biological processes[25].

It is, in many ways, remarkable that this degree of heterogeneity in the routes to cancer can lead to such convergent phenotypes. Although detailed genotype–phenotype studies in the massively parallel sequencing era are lacking, it is nonetheless the case that, for example, a histologically typical ER-positive breast cancer can result from a wide array of different cancer genes that have been mutated through many different processes. It is also conceivable that diverse sets of genes will also give rise to cancers with similar behaviours and sensitivities to certain treatments. Optimizing the clinical benefit of cancer genomics for the future therefore demands the systematic integration of genomic data with meaningful clinical information in large databases.

Heterogeneity within an individual cancer

A number of external forces can act on the cancer genome to generate heterogeneity and to influence the subclonal structure (FIG. 2). The tumour microenvironment has an important role in selecting the cells that are best adapted to the (often hostile) environments in which they exist[46]. The identification of organ-specific branches within phylogenetic trees in

metastatic studies is also indicative of environmental factors that select and drive specific genomic changes[11,12] (BOX 1). Carcinogenic exposures, such as tobacco smoke, ultraviolet light and even some cancer treatments, may also have an important direct role in driving cancer heterogeneity[6,45,47–49]. The selective 'environment' also includes anticancer treatments. For example, verumafenib — an inhibitor of the serine/threonine protein kinase BRAF — has revolutionized the treatment of metastatic melanoma by providing a targeted therapy for patients with the V600E *BRAF* point mutation. However, patients usually relapse within a few months as a result of emerging resistance. It is postulated that resistant clones are selected on the basis of either pre-existing or *de novo* abnormalities arising in alternative pathways[50,51]. This implies that genomic heterogeneity supports cancer survival in response to a changing environment.

Cellular ground state and cancer evolution

The observation that specific genes are associated with certain types of cancer in some tissues but not others indicates that the cell of origin may be an important factor in dictating the evolutionary trajectory. Every cell in the body is clonal, having arisen from a single zygote. There is some 'physiological' genomic change within organ systems, such as rearrangement and mutation of immunoglobulins in lymphocytes and somatic retrotransposition of long interspersed elements (LINEs) in the brain[52], but it is largely the case that the huge phenotypic variability among cells in a human is dictated by the epigenome, transcriptome and proteome of those cells. It follows that this 'ground state' of a cell in which a somatic mutation arises will strongly influence how that mutation plays out, as the early life of a somatic mutation is fraught with the threat of extinction through random genetic drift. This concept is exemplified by the *BCR–ABL* fusion gene, which is frequently associated with a range of haematological malignancies. Studies have identified that the activation of ABL kinases in breast cancer cell lines promotes invasiveness; however, *BCR–ABL* has not been implicated in the pathogenesis of solid tumours[53]. Reasons for this specificity may include the low transcriptional activity of the *BCR* promoter in non-haematological cells, a lack of interacting partners needed for full oncogenic effects of the fusion protein or failure to induce a sustaining population of cancer cells.

The importance of the 'ground state' is exemplified by the specific 'oncogenicity' of *KIT* mutations in gastrointestinal stromal tumours (GISTs). KIT is a receptor tyrosine kinase that is activated by stem cell factor binding (also known as mast cell growth factor binding), resulting in a signalling cascade that promotes cell survival, differentiation and proliferation, and germline *KIT*-activating mutations are associated with hyperplasia of interstitial cells of Cajal (ICCs) and GISTs[54]. In mouse models, it has recently been demonstrated that GISTs exclusively arise in a subset of ICCs that expresses high levels of endogenous ETS variant 1 (*ETV1*)[55,56]. ETV1 is a member of the ETS family of transcription factors, which are involved in various key cellular processes, including cell cycling, proliferation and differentiation. In ICCs, ETV1 acts as both a survival factor and as a master regulator of a specific transcription programme that is co-opted by and required for transformation by activated KIT[56]. The implication is that in the absence of high levels of endogenous ETV1 expression, *KIT* mutations fail to drive the emergence of GIST cancers.

However, in most situations, the link between the cell of origin and the cancer phenotype appears to be less clear-cut. As an increasing number of genomic studies report broad catalogues of cancer genes, it is becoming apparent that many of the same genes are implicated across a broad range of tissue types — albeit at different frequencies. For example, two independent studies identified that cancer genes that are historically associated with haematological malignancy, such as runt-related transcription factor 1 (*RUNX1*) and core-binding factor, beta subunit (*CBFB*), are also recurrently mutated in breast cancer[30,57].

The role of epistasis in cancer genome evolution

A new mutation in a cancer gene does not occur in isolation but rather enters into an established genomic landscape. This existing gene network may have a profound effect on the fate of the cell, determining whether there is a cell death or clonal expansion. The ground state of a cell can be considered to represent interactions with cellular identity, whereas epistasis, by contrast, represents interactions among oncogenic mutations.

Three major lines of evidence drawn from recent studies have demonstrated the probable importance of epistatic factors in cancer genome evolution. First, the large cancer gene databases have shown that, despite extensive heterogeneity in common cancers, particular combinations of somatic mutations may co-occur more than expected by chance, such as *TP53* and breast cancer 1, early onset (*BRCA1*) and breast cancer 2, late onset (*BRCA2*) mutations in breast cancer[58] or the oncogenic *KRAS* and serine/threonine kinase 11 (*STK11*; also known as *LKB1*) mutations in lung cancer[59,60]. Second, activation of many oncogenes, including *KRAS*, can lead to a state of 'oncogene-induced senescence'[61,62]. This is an acute and telomere-independent form of senescence that can occur in response to the expression of oncogenes and is protective against cancer. It is widely believed that second hits, such as cyclin-dependent kinase inhibitor 2A (*CDKN2A*) inactivation, are required to ameliorate these effects[63,64]. Third, convergent evolution among subclones within the malignant tumour (or tumours) of a particular patient also implies cooperativity among somatic mutations[12,15,65], and this is exemplified by patients with renal cancer[9] (BOX 1). A recent multi-region sampling study identified that after ubiquitous von Hippel–Lindau tumour suppressor, E3 ubiquitin protein ligase (*VHL*) loss, driver mutations inactivating histone modifiers can independently arise in different branches of the phylogenetic tree. Even more strikingly, independent phosphatase and tensin (*PTEN*) mutations occurred twice in one patient in different subclones, despite *PTEN* mutations being found in only 1% of renal cancers overall[9]. This implies that some specific feature of the genomic landscape of this patient's cancer was particularly dependent on the inactivation of *PTEN* — an event that is not required for most renal cancers.

Little is known about whether the order of mutation acquisition is important. The renal cancer studies described above do suggest a pre-requisite for early *VHL* loss in renal cancer, but the extent to which this is a general rule is unclear. Ancestral gene reconstruction and protein-engineering studies demonstrate that epistatic interactions can limit the potential mutational trajectories that are available and can also enforce ratchet-like constraints by inhibiting the reversibility of the evolutionary process[66]. Certain oncogenic mutations may mandate that specific cellular pathways be targeted by subsequent mutations. Such an effect would restrict the set of potential driver mutations that could occur after the initial event[67].

The strands of data discussed above imply cooperativity among cancer-causing mutations, and this cooperativity can include mutations that ameliorate negative effects of other variants (synthetic viability) or mutations that, when combined, result in synergistic effects (greater than the sum of their individual effects). This is exemplified by the interaction between the proto-oncogene *MYC* (also known as *c-MYC*) and B cell CLL/lymphoma 2 (*BCL2*) in cell lines. The overexpression of MYC induces apoptosis, but the co-expression of BCL2 overrides this effect and permits MYC to drive the cell into cycling[68,69].

From the clinical standpoint, discovering and understanding epistatic interactions such as synthetic lethality is proving useful in the design of targeted therapies[70]. The sensitivity of $BRCA1^{-/-}$ cells to poly(ADP-ribose) polymerase (PARP) inhibitors is already a widely cited 'synthetic lethality' interaction in the clinic[71]. BRCA1 is essential for homologous recombination repair of dsDNA breaks. $BRCA1^{-/-}$ cells are able to survive despite this defect, but it comes at the cost of critical dependence on alternative repair pathways

involving PARP function. Therefore, in *BRCA1*$^{-/-}$ cells that have been treated with PARP inhibitors, DNA breaks that arise from collapsed replication forks cannot be repaired, resulting in cell arrest and death. However, in the face of PARP inhibitor therapy, 'reversion' mutations in *BRCA1* and *BRCA2* can result in the restoration of a partially functional protein homologous recombination[72,73], leading to the escape of the clone from the detrimental effects of the treatment. This example shows the clinical potential to exploit epistatic interactions but also the complexity of these networks and the problems posed by the dynamic and rapidly evolving cancer genome.

The role of genomic crises in tumorigenesis

Recent lines of evidence derived from directly studying cancer genomes indicate that, in some cases, a huge number of mutations can occur in a timescale that is considerably shorter than that on which clonal selection operates (FIG. 3). These mutational processes can take several forms.

Telomere attrition is associated with end-to-end chromosome fusions, and this can drive massive genomic disruption through repeated breakage–fusion–bridge cycles[74]. An end-to-end chromosome fusion generates a dicentric chromosome (that is, a chromosome with two centromeres), and the two centromeres are pulled to opposite daughter cells during mitosis, generating further DNA breaks. This process can be repeated with every cell cycle until a telomere is restored to the naked DNA ends. Within a few cell cycles, and certainly on a much faster timescale than natural selection can operate, widespread chromosomal deletions and exponential genomic amplification can develop[75,76].

Balanced chains of somatically acquired genomic rearrangements have been observed in prostate cancer[40] and some haematological malignancies. These chains can show up to ten genomic regions involved in a mutual exchange of DNA segments without copy number loss. In some cases, these generate oncogenic fusion genes (for example, in the *TMPRSS2–ERG* loci) or gene disruptions. Curiously, regions that are involved in these chains show a propensity to involve highly transcribed genes. In one example, breakpoints were in close proximity to four potential cancer genes: TANK-binding kinase 1 (*TBK1*), *TP53*, mitogen-activated protein kinase kinase 4 (*MAP2K4*) and *ABL1*.

Approximately 2–3% of cancers show evidence for a catastrophic mutational process that has been coined chromothripsis[41]. A process of genome shattering and reassembly occurs in a one-off crisis, resulting in a characteristic pattern of oscillating DNA copy number and up to several hundred genomic rearrangements localized to one or a few chromosomes. This localization may be the result of physically isolating the damaged chromosomes in micronuclei created during anaphase[77]. Chromothripsis has been observed at a low frequency in a diverse range of cancers, including chronic lymphocytic leukaemia (CLL), neuroblastomas, myelomas, breast cancer, small and non-small-cell lung cancers and renal, thyroid and gastrointestinal malignancies[8,41,43]. It is notable that a recent study identified high rates of chromothripsis in medulloblastomas and AML in the presence of mutant *TP53* (100% and 47% of cancers, respectively) but not wild-type *TP53* (0% and 1%, respectively)[42]. Bone sarcomas also seem to have a particularly high rate of chromothripsis[41].

In addition to clusters of structural variants, multiple point mutations can also be acquired in one-off bursts. In a sizable proportion of breast cancers, we have observed clusters of cytosine mutations near sites of genomic rearrangement: a process that we termed kataegis[24]. These clusters can represent up to 10–20 base substitutions in one or two kilobases, all occurring at cytosines in a TpC context, all collinear (that is, in linkage) and all occurring on either the forward or reverse strand of DNA. The mechanism underlying such

events remains mysterious, although the mRNA-editing APOBEC proteins may be involved[25]. This phenomenon has not yet been reported in other types of cancer.

All of these catastrophic mutational processes imply that cancer genome evolution may not always be a gradual stepwise progression (FIG. 3). In one of the patients with chromothripsis, the crisis simultaneously disrupted three tumour-suppressor genes — the E3 ubiquitin protein ligase *FBXW7*, *CDKN2A* and the RecQ helicase *WRN*[41]. This suggests that the clone would have taken a substantial leap along the path to malignancy after the catastrophic event. In multiple myelomas, samples with evidence for chromothripsis were associated with reduced survival, indicating that the large-scale genomic disruption may have rendered the myeloma cells more malignant, with similar data emerging for neuroblastoma[78,43]. These mechanisms of mutation accumulation are not mutually exclusive. Gradual mutation accumulation occurs to some degree in all cancers, representing perpetual adaptation to the tumour environment but may be punctuated by highly disruptive episodes.

The role of mutation rate in cancer evolution

The number of driver mutations required for a cancer to become fully malignant is debated, but it is generally considered to be between 2 and 20 in most types of common solid malignancy[79,80]. Some cancers, such as certain subtypes of AML, accumulate a sufficient complement of mutations to transform to a malignant phenotype in the presence of an apparently normal mutation rate[81]. There are several lines of evidence, however, indicating that many cancers achieve the required complement of driver mutations by means of an elevated mutation rate.

‘Mutator mutations’ in carcinogensis

‘Mutator mutations’ are mutations within cancer genes that increase the mutation rate across the cancer genome. The effects of such mutations can be broadly categorized as: reduced ability to detect and/or repair DNA damage; failure of genomic surveillance mechanisms; and increased susceptibility to DNA damage by exogenous and endogenous carcinogens (FIG. 4). It has long been recognized that inherited cancer syndromes, including ataxia telangiectasia, xeroderma pigmentosum, Bloom’s syndrome and hereditary non-polyposis colorectal cancer, are caused by germline defects in specific DNA repair genes[82]. They are associated with an elevated mutation rate and are characterised by early onset cancers.

Microsatellite instability (MSI) is characterized by a high rate of substitutions and small insertions and deletions, and it arises from mutations in mismatch repair genes, including *MSH2* and *MCH1* (REF. 44). MSI occurs in less than 20% of colorectal cancers and has also been reported at low frequency in a diverse range of other tumour types including gastric, endometrial and sebaceous cancers and lymphomas[83]. A much more frequent pattern of genomic instability that is seen in nearly all types of common solid malignancy, including breast and colorectal cancer, is chromosomal instability (CIN), which is a process whereby whole-chromosome segregation abnormalities during mitosis result in aneuploidy[84–86].

Epigenetic instability is also common in a wide range of cancers (BOX 3). Aberrant methylation of CpG islands in promoter regions is correlated with silencing of multiple tumour suppressor genes, resulting in the CpG island methylator phenotype (CIMP). This has been observed in many cancer types and is associated with aetiologically and clinically distinct types of colorectal cancer[87] and glioma[88].

Elevated mutation rate

Many of the aggressive clinical characteristics of cancers, such as the abilities to resist treatment, to relapse and to metastasize, depend on the continued generation of genetic variation that permits adaptation[7,12]. However, whether all cancers have elevated mutation rates compared to normal cells has been controversial[81,89,90]. In the era of whole-cancergenome sequencing, however, it is becoming clear that the overwhelming majority of tumours carry hundreds to hundreds of thousands of somatic mutations, which is suggestive of an elevated mutation rate.

A preponderance of a specific type of base substitution in a given context, such as C-to-T mutations in a CpG context, can be viewed as a 'mutational signature' that reflects an underlying mutational process[6,12,45]. Analysing these mutational signatures in 21 breast cancer genomes identified several major processes involving base substitutions, small insertions and deletions and genomic rearrangements[25]. Mutations acquired early in the development of the cancer were dominated by C-to-T transitions, especially in a CpG context, that are likely to represent spontaneous deamination of methylated cytosines. This is a rather generic mutational process, is similar to that seen in the germ line and reflects that seen by exome sequencing of normal haematopoietic stem and/or progenitor cells in healthy people[81]. However, nearly all tumours demonstrated a substantial shift in the contribution from individual processes over time with several novel mutational processes reported generally emerging late in the development of the cancer[24]. Taken together, this information implies that the vast majority of breast cancers have an elevated, cancer-specific increase in mutation rate. Environmental exposures, including the traditional cancer treatments (namely, chemotherapy and radiotherapy), also influence mutation rate and spectrum. Chemotherapy, for example, is associated with an increase in transversions[21]. The functional effect of endogenous and external factors that increase mutation rate on cancer progression remains to be elucidated.

There may be examples of tumours in which mutation rate per cell division is not increased. AML, for example, does not have an excessive mutation burden, and when compared with age-matched normal haematopoietic cells, mutation numbers are broadly similar[81]. In a similar fashion, there is a correlation between age and mutation burden in the childhood tumour, medulloblastoma[34,91], suggesting that mutation accumulation in this disease is more a function of time than it is an acquisition of specific mutational processes.

Mutation rate distribution across the cancer genome

Mutation is generally modelled as a random process, but there is increasing evidence that the distribution of somatic mutations shows variegation across the genome in both the rate and the type of variation. The most extreme example of this is somatic hypermutation in lymphoid malignancies. In normal B lymphocyte ontogeny, the immunoglobulin gene is subjected to targeted mutation to increase antibody diversity in response to infection. Sometimes, however, the tightly controlled genomic localization of the hypermutation machinery can be loosened, and other genes that are highly expressed during lymphoid differentiation may be subjected to this process. This aberrant somatic hypermutation has only been described in association with a handful of genes. It preferentially targets the 5′ untranslated region and the first coding exon of the gene and can repeatedly occur during lymphoma development, driving much subclonal diversity just at these specific loci[92]. In particular, the oncogene *BCL6* is commonly mutated in this way in diffuse large B cell lymphoma[93,94].

Less extreme examples of variable mutation rates across the genome abound. Chromosomal fragile sites have been documented in cytogenetic studies for some years, and cancers show

increased rates of heterozygous and homozygous deletion at these sites compared to other regions of the genome[95,96]. This increased rate of genomic rearrangement may in part result from these regions having fairly sparse origins of replication and being late replicating during the cell cycle[97,98]. In many cases, these deletions are of no biological consequence to the cell, but there is some evidence that cancer genes may reside in these loci. For example, Parkinson protein 2, E3 ubiquitin protein ligase (*PARK2*) can be recurrently deleted and mutated in gastrointestinal tumours[99], and knockout of the gene in mice increases the rate of *APC*-induced colorectal tumours[100].

The underlying chromatin state may also contribute to genomic rearrangement. In lymphomas, genes that are frequently fused with the immunoglobulin locus are often geographically proximate during interphase[101], and in prostate cancer androgen can induce intra- and interchromosomal proximity between the *ETS* fusion gene partners[102,103]. In breast cancer, 50% of somatically acquired genomic rearrangements involve a gene footprint compared with 40% expected by chance[104].

With regard to point mutations, mismatch repair deficiency causes a specific distribution and signature of mutations across the genome. For example, in microsatellite-unstable colorectal cancer, genes such as the type 2 TGFβ receptor gene are particularly prone to mutation owing to their specific nucleotide mix, whereas this gene is almost never mutated in microsatellite-stable colorectal cancer[105]. Similarly, the distribution of oncogenic point mutations in *TP53* and *KRAS* in tobacco-induced lung cancer differs from lung cancers that develop in people who have never smoked[45,49]. The efficacy of DNA repair processes also leads to genomic variation in mutation rate: in carcinogen-induced tumours especially, lower rates of mutation are seen in highly expressed genes compared with non-expressed genes[106].

How does this variegation in mutation rate across the genome impact on our understanding of cancer evolution? Clearly, cancer can arise from a vast array of different possible driver mutations. These data indicate that the observed distribution of driver mutations seen in a given tumour type depends not only on the oncogenicity of the given genes in that cellular context (namely, the selective advantage associated with the mutation) but also on the probability with which a given change can arise in the population of competing clones.

Conclusions

In the not too distant future, genomic features of every patient's cancer type will be characterized at the point of diagnosis. A list of implicated cancer genes and mutational processes will be generated, and a personalized therapeutic regimen will be chosen. One of the major challenges to this vision is how to sample the cancers to attain an accurate view of the underlying complexity and to address the fact that cancers are highly dynamic evolutionary processes[9]. A single sample is a 'snapshot' in space and time. Multi-region sampling and sampling of distinct metastatic sites will help to reduce the problem posed by geographical heterogeneity but will have to be balanced with clinical risk and patient choice. It is necessary to acknowledge that even with the most sensitive and accurate of genomic technologies, clinically important mutations that are confined to subclones may be missed on account of inadequate sampling. The clinical approach towards sampling will therefore be guided by multi-sampling studies within all cancer types, and in particular important insights may be gained from studies that use sequential time-ordered sampling of cancers with well-defined precursor lesions, such as cervical intra-epithelial neoplasia in cervical cancer and Barrett's oesophagus in oesophageal cancer.

Understanding how the cancer genome responds to treatment and promotes metastasis presents a further challenge, requiring longitudinal sampling strategies incorporated into

long-term clinical trials. Furthermore, the optimal targeted therapeutic approaches to cancers with branching evolutionary architectures remains unclear. The observation that any individual cancer may contain both clonal driver mutations (that is, mutations that occur within the phylogenetic tree trunk) and subclonal driver mutations, which are linked through epistatic interactions, indicates that cancer eradication may well demand complex combinations of drugs.

Finally, the heterogeneity of cancer genes and cancer pathways mutated across human malignancy mandates the development of large-scale, publicly available databases with carefully annotated clinical outcome data linked to detailed genomic analyses. After sample sizes in these databases have reached numbers in the tens of thousands, we will have the raw material with which to build algorithms for personalized decision support for oncologists.

Acknowledgments

We would like to thank the Wellcome Trust for funding.

Glossary

Mutational signatures	Patterns of mutations that are characteristic of a type of cancer or that are indicative of a specific process.
Chromothripsis	A single event that causes genome shattering and reassembly, resulting in a characteristic pattern of oscillating copy number and up to several hundred genomic rearrangements localized to one or a few chromosomes
Driver mutations	Somatic mutations within cancer genes that confer a clonal advantage, that are causally implicated in oncogenesis and that are positively selected for during cancer evolution.
Synthetic lethality	Two genes are synthetically lethal if mutation of either in isolation is compatible with viability, but mutation of both to cell death.
Kataegis	A localized hypermutation that often colocalizes with somatic rearrangements.
Microsatellite instability	(MSI). Microsatellites are repeating sequences within DNA of 2–6 base pairs in length; defects in mismatch repair can give rise to genomic instability within these regions.
Chromosomal instability	(CIN). A form of genomic instability that is common in cancers and is characterized by large chromosomal losses by as of yet undefined mechanisms.

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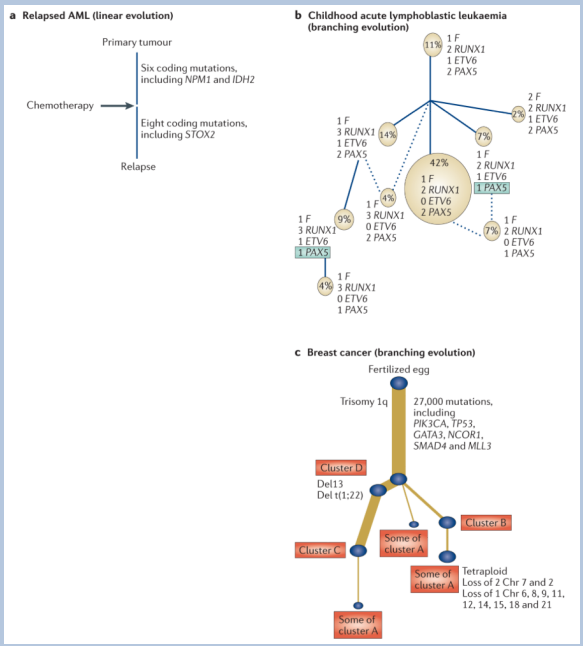
Box 1

Phylogenetic cancer trees



A phylogenetic tree is a pictorial representation of how a tumour is inferred to have evolved. As discussed in the text, these inferences can be based on a wide range of molecular biology and sampling techniques coupled with existing and new bioinformatics algorithms for reconstructing the tree. Several key properties of the evolution of a tumour are coded in the tree and provide important biological information about the genetic diversity of a cancer and clonal mix.

All trees have a shared ‘trunk’, which represents the complement of mutations shared by all malignant cells within the cancer. Because these mutations are fully clonal, there must have been a single ancestral cell that carried all of these mutations and through which all extant tumour cells can trace their lineage; we denote this cell the ‘most recent common ancestor’, borrowing the term from population genetics. Emergence of this cell initiated the final complete selective sweep within the cancer: all clonal expansions thereafter are, by definition, incomplete. All mutations that occur after the most recent appearance of a common ancestor are subclonal.

The length of individual branches (and the trunk) denotes the number of mutations that occurs in that lineage: a so-called ‘molecular clock’. If mutation rates per unit time were constant, then this would correlate with chronological time. However, for many cancers, this assumption is probably invalid (as discussed in the text), and molecular time is likely to be a poor proxy for chronological time.

The branching structure of the tree captures the number of subclonal populations within the cancer samples and their genetic relationships. For example, both linear and branching patterns of evolution have been described in a range of cancers. Linear evolution (panel **a** of the figure) was described in acute myeloid leukaemia (AML) and identifies the post-treatment relapse clone as a direct descendant of the major clone. The tree in panel **b** demonstrates branching evolution and specifically convergent evolution, in which the same genetic consequence independently emerges in separate clades of the phylogenetic tree highlighted by green boxes containing recurrently mutated genes.

Brown circles represent cytogenetically distinct populations, and the numbers represent the number of copies of each adjacent gene. Solid lines represent the most likely ancestral origin of subclones, whereas dashed lines suggest alternative origins.

As sequencing goes genome-wide, phylogenies have been constructed for single-tumour samples that are composed of multiple constituent cellular subclones. The identification of tens of thousands of mutations genome-wide permits the delineation of distinct clusters of mutations — these clusters consist of groups of mutations that share similar mutant allele frequencies (corrected for local copy number). In the tree in panel **c**, we present a phylogenetic tree in which the variable thicknesses of the branches reflect the numbers of mutations within each distinct mutation 'cluster'. This gives an indication of the patterns of subclonal importance and dominance within the cancer population. Chr, chromosome; *ETV6*, ETS variant 6; *F*, *ETV6–RUNX1* fusion gene; *GATA3*, GATA-binding protein 3; *IDH2*, isocitrate dehydrogenase 2; *PAX5*, paired box 5; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; *NCOR1*, nuclear receptor co-repressor 1; *MLL3*, myeloid/lymphoid or mixed-lineage leukaemia 3; *NPM1*, nucleophosmin (nucleolar phosphoprotein B23, numatrin); *RUNX1*, runt-related transcription factor 1; *SMAD4*, SMAD family member 4; *STOX2*, storkhead box 2. Panel **a** is adapted, with permission, from REF. 21 © (2012) Macmillan Publishers Ltd. Panel **b** is adapted, with permission, from REF. 15 © (2011) Macmillan Publishers Ltd. All rights reserved. Panel **c** is adapted, with permission, from REF. 24 © (2012) Cell Press.

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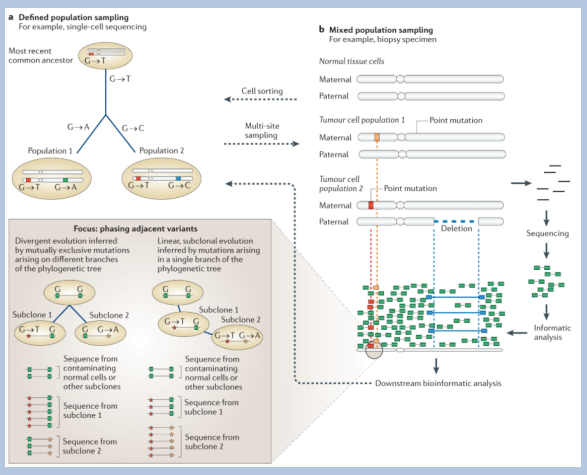
Box 2

Methodologies for understanding cancer genome evolution



The existing methods used to hypothesize the order of evolutionary events, or phylogeny, derive from molecular genetics. These methods use multiple sampling techniques and assume that individuals can be isolated in a population. The evolutionary connections between organisms are calculated using a range of mathematical models, including parsimony, maximum likelihood, Markov chain Monte Carlo and Bayesian inference. Cancer genomics has adopted these tools to reconstruct the relationships between mixed populations of cells in individual cancers[10,13–15]. This approach is suitable when individual subclonal populations in a cancer can be reliably isolated, such as through single-cell sequencing or cytogenetics. A simplified example of this is represented in panel **a** of the figure, in which two different mutations result in evolutionary divergence from a presumed most recent common ancestor.

However, as illustrated in panel **b** of the figure, most cancer samples consist of mixed populations of normal cells and tumour cells, and next-generation sequencing data therefore provide a composite view of a random sample of DNA molecules from these different populations. Mutations in the data follow defined probability distributions that are dictated by coverage and the underlying allele frequency. There remains a paucity of statistical algorithms for analysing these data, but some useful techniques have recently been developed, such as mutational clustering, using kernel density analysis[21] and Bayesian Dirichlet process modelling[24,107], digital karyotyping[26] and phasing adjacent somatic mutation pairs or adjacent somatic mutations and germline variants[24]. The phasing technique is summarized in the focus box (panel **b**).

The above methodologies may be amalgamated to handle the data from multi-site sampling studies that include defined populations that are nonetheless genetically heterogeneous[7,9,12].

Box 3

The interplay of the epigenome and genome in cancer evolution by natural selection

For epigenetic factors to be important in cancer evolution, three criteria must be met: stochastic variation must exist among competing clones in a given epigenetic locus; this variation must be heritable; and there should be phenotypic consequences of epigenetic variation for natural selection to act on.

Within cancers, individual tumour suppressor genes may be epigenetically silenced through promoter CpG island hypermethylation[108]. However, in contrast to mutation, little is known about the stochastic acquisition and loss of epigenetic changes. Nonetheless, bisulphite sequencing of individual haplotypes of CpGs has shown that some regions do show variability across different cells in a sample[109,110]. There is a robust machinery of cytosine methylases that faithfully copy methylation at CpG dinucleotides from the template strand to the newly synthesized DNA strand during DNA replication, indicating that these changes are heritable. It is less clear how histone marks are transmitted to the daughter cells, although such pathways are presumed to exist.

An emerging theme of recent genomic discoveries in cancer has been the frequent mutation of genes that are involved in epigenetic regulation, further highlighting the importance of interactions between genetic and epigenetic changes. This is exemplified by mutations in AT-rich interactive domain 1A (*ARID1A*) in ovarian cancer[111,112], inactivation of polybromo 1 (*PBRM1*), lysine-specific demethylase 5C (*KDM5C*), *KDM6A* (also known as *UTX*) and SET domain containing 2 (*SETD2*) in renal cancer[113] and the remarkable observation of activating mutations of the Polycomb group gene *EZH2* in follicular lymphoma[54] but inactivating mutations of the same gene in chronic myeloid malignancies[114,115]. Chromatin studies have indeed shown epigenetic consequences of these mutations, but we lack a detailed understanding of the particular target genes involved or the Darwinian evolution of the epigenetic landscape after these genomic aberrations appear. Nonetheless, a recent genome-wide methylation profiling study in acute myeloid leukaemia identified that genetically distinct subtypes of disease carried characteristic epigenetic profiles[116]. This implies that a particular driver gene may promote the evolution of and may cooperate with an epigenetic landscape that is ‘optimal’ for that genomic change.

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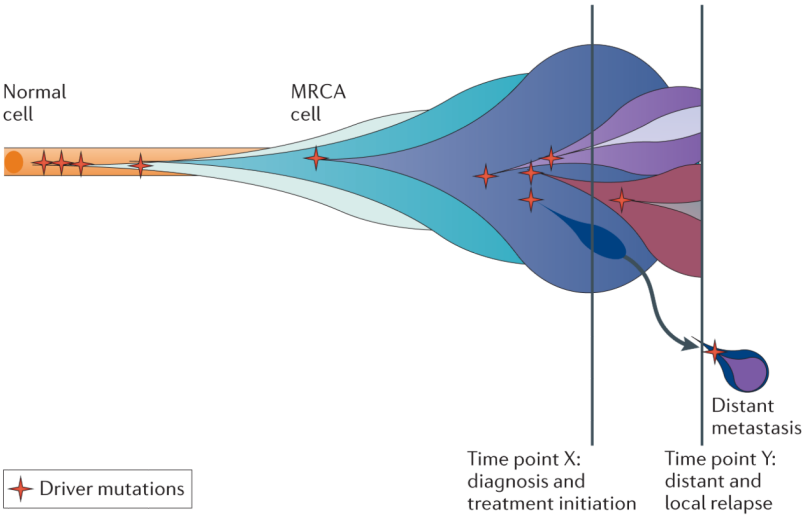

Figure 1. The evolution of clonal populations

Cancers are genomically diverse and dynamic entities. Unique clones (represented by different coloured bubbles) emerge as a consequence of accumulating driver mutations in the progeny of a single most recent common ancestor (MRCA) cell. Ongoing linear and branching evolution results in multiple simultaneous subclones that may individually be capable of giving rise to episodes of disease relapse and metastasis. The dynamic clonal architecture is shaped by mutation and competition between subclones in light of environmental selection pressures, including those that are exerted by cancer treatments.

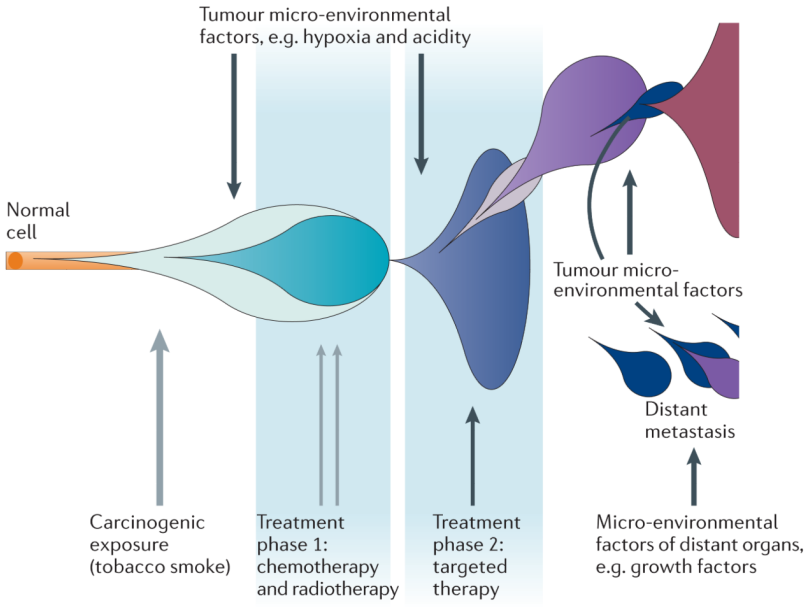

Figure 2. The role of the environment in evolutionary adaptation

A multitude of environmental factors may shape the evolutionary processes within a single cancer. Blue and purple bubbles represent successive cancer clones, the expansion of which is altered by directly mutagenic factors (grey arrows) and non-mutagenic factors (black arrows).

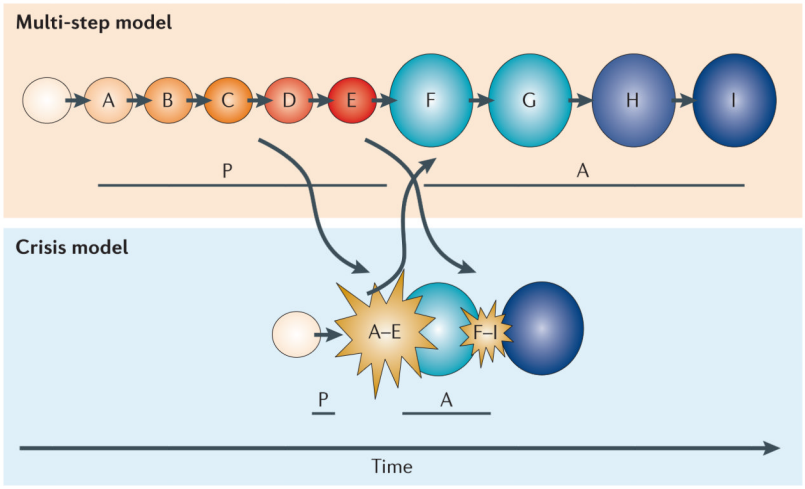

Figure 3. Stepwise versus crisis-driven mutation accumulation

Multi-step and crisis event models of carcinogenesis are represented. It is thought that these pathways are not necessarily mutually exclusive but that they may coincide and overlap. In this example, mutations A–E (orange to red circles) are those that are required to initiate clonal expansion and malignant transformation, whereas mutations F–H (blue circles) drive ongoing evolution and the acquisition of aggressive clinical characteristics. The pre-malignant phase (P) and the time from malignancy onset to acquisition of an aggressive phenotype (A) are reduced in the crisis event model compared to the multi-step model. This indicates that standard screening techniques that aim to detect pre-invasive and early malignancies may be inadequate in cancers that develop through crisis events.

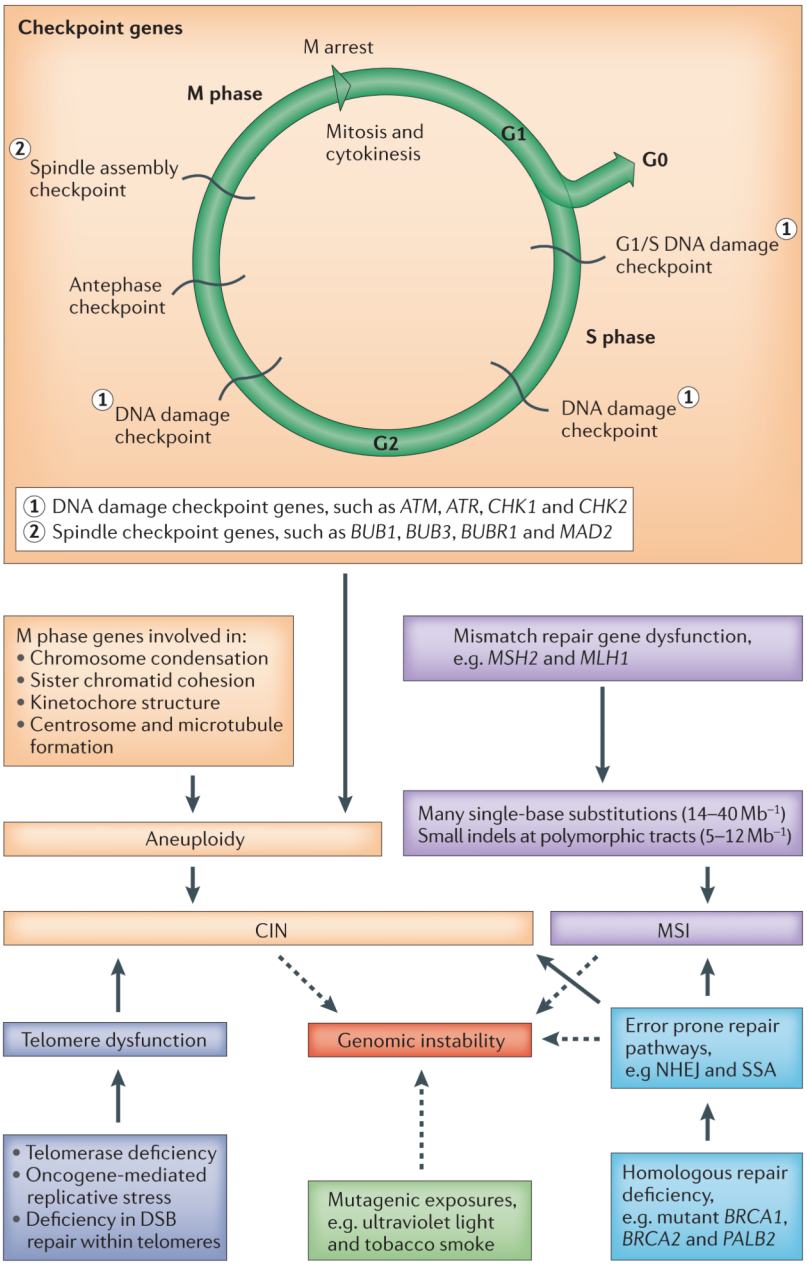

Figure 4. 'Mutator mutations' drive genomic instability in cancers

There are two major recognized routes by which genomic instability may arise. Chromosomal instability (CIN) is common across all types of cancer and may be numeric (aneuploidy) or structural. CIN may arise through mutations in a wide range of genes involved in cell cycling and division (orange boxes) or through other diverse mechanisms, such as telomeric dysfunction or as a consequence of failure in homologous repair. Microsatellite instability (MSI) is less common and occurs as a result of mutations in the mismatch repair genes (purple boxes). Instability may also directly arise as a consequence of defects in homologous repair, necessitating the use of alternative error prone pathways, such as non-homologous end joining (NHEJ) and single-strand annealing (SSA). Error-prone pathways may result in both chromosomal instability and genomic instability through

frequent small deletions or substitutions. Mutagenic exposures may also contribute to genomic instability. *ATM*, ataxia telangiectasia mutated; *ATR*, ataxia telangiectasia and Rad3-related; *BUB1*, budding uninhibited by benzimidazoles 1; *BUBR1*, budding uninhibited by benzimidazoles 1 beta; *BRCA1*, breast cancer 1, early onset; *BRCA2*, breast cancer 2, early onset; DSB, double-strand break; indel, insertion or deletion mutation; *MAD2*, MAD2 mitotic arrest deficient-like 1; *MSH2*, mutS homologue 2, colon cancer, nonpolyposis type 1; *MLH1*, mutL homologue 1, colon cancer nonpolyposis type 2; *PALB2*, partner and localizer of *BRCA2*.

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