Mechanisms underlying mutational signatures in human cancers

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Abstract | The collective somatic mutations observed in a cancer are the outcome of multiple mutagenic processes that have been operative over the lifetime of a patient. Each process leaves a characteristic imprint — a mutational signature — on the cancer genome, which is defined by the type of DNA damage and DNA repair processes that result in base substitutions, insertions and deletions or structural variations. With the advent of whole-genome sequencing, researchers are identifying an increasing array of these signatures. Mutational signatures can be used as a physiological readout of the biological history of a cancer and also have potential use for discerning ongoing mutational processes from historical ones, thus possibly revealing new targets for anticancer therapies.

Driver mutations
Genetic changes that give selective advantages to clones during cancer development.

Somatic mutations
Mutations that are acquired as opposed to inherited.

Passenger mutations
Genetic changes that do not confer any selective advantage in cancer development.

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Until recently, cancer research was focused on the discovery of driver mutations (that is, key somatic mutations that are causally implicated in oncogenesis and that confer selective advantages during the evolution of a cancer)[1]. However, a cancer contains more than a mere handful of driver mutations. Each cancer bears many thousands of passenger mutations that may not be causative of cancer development but that are a rich source of historical information[1–3]. Although they are not the focus of positive selection, these bystander mutations are the product of, and therefore bear the 'scars' of, the biological perturbations (that is, the mutational processes) that have occurred throughout the development of a cancer[1–3]. Each mutational process leaves a characteristic pattern — a mutational signature — on the cancer genome, which is defined by the type of DNA damage that has occurred as a result of a plethora of exogenous and endogenous DNA damaging agents, as well as by the DNA repair or replicative mechanisms that were successively activated. Irrespective of the nature of these mutagenic or repair mechanisms, the final catalogue of mutations is also determined by the strength and duration of exposure to each mutational process[2] (FIG. 1). Additionally, cancers are likely to comprise different cell populations (that is, subclonal populations), which can be variably exposed to each mutational process; this promotes the complexity of the final landscape of somatic mutations in a cancer genome[3]. The final mutational portrait, which is obtained after a cancer has been removed by surgery and then sequenced, is therefore a composite of multiple mutational signatures (FIG. 1).

The advent of next-generation sequencing technology[4] has led to an extraordinary surge in the speed and scale of sequencing[5,6]. Large-scale sequencing of all protein-coding exons (using whole-exome sequencing) or even whole cancer genomes (using whole-genome sequencing) is achievable in a single experiment[7,8]. These sequencing efforts yield many thousands of mutations per cancer and thus provide sufficient power to detect mutational signatures. Mathematical algorithms can then be applied to these big, complex and multidimensional data sets to extract individual mutational signatures[9,10] and to quantify these in the cancer of each patient[2,9,10] (BOX 1). The number of mutations that contribute to each signature is a proxy for the amount of exposure to each mutational process, which can vary considerably from one cancer to another. Mutational signatures therefore provide an account of not only the mechanism that has gone awry in the cancer cell but also the degree to which it has been affected by this perturbation. Nevertheless, in-depth knowledge of the underlying individual mutational processes is still lacking. A better understanding of how particular mutational signatures arise is important in order to distinguish ongoing mutational processes from historical ones (FIG. 1). Historical mutational processes are informative of past exposures, and mutational signatures that underlie these processes therefore have an important message regarding cancer prevention and public health. However, they have limited value as biomarkers or therapeutic targets, as they are no longer actively promoting cancer development. By contrast, ongoing mutational processes

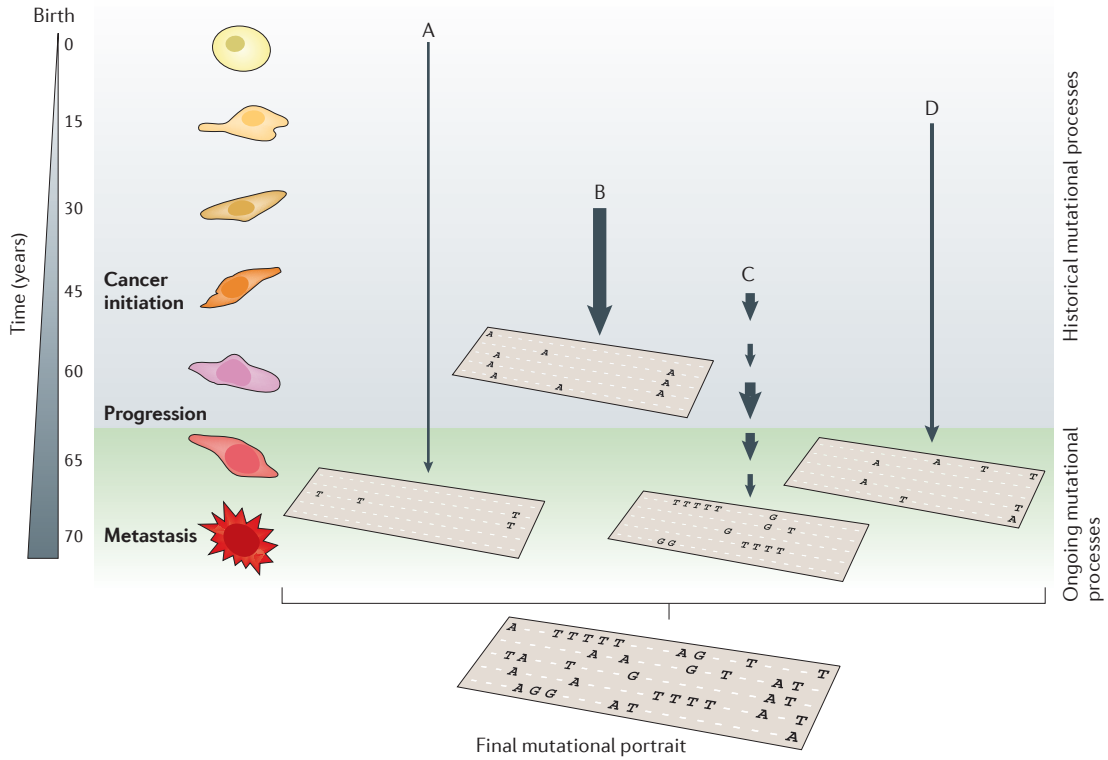


Figure 1 | **Active mutational processes over the course of cancer development.** Each mutational process leaves a characteristic imprint — a mutational signature — in the cancer genome and comprises both a DNA damage component and a DNA repair component. In this hypothetical cancer genome, arrows indicate the duration and intensity of exposure to a mutational process. The final mutational portrait is the sum of all of the different mutational processes (A–D) that have been active in the entire lifetime. Ongoing mutational processes reflect active biological processes in the cancer that could be exploited either as biomarkers to monitor treatment response or as therapeutic anticancer targets. By contrast, historical mutational processes are no longer active. Signature A represents deamination of methylated cytosines, which is ongoing through life. Signature B can be matched up with the signatures of tobacco smoking, Signature C can represent bursts of APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide)-induced deamination, and Signature D represents a DNA repair pathway that is awry.

Mutational processes
Biological activities that generate mutations; each of these processes comprises both a DNA damage component and a DNA repair component. These processes can be ongoing or historical depending on whether the biological processes that cause the acquisition of mutations in a cancer are active or inactive, respectively.

Mutational signature
The pattern of mutations produced by a mutational process.

Mutational portrait
The total genetic changes observed in a cancer genome; that is, the sum of all mutational signatures occurring in a lifetime.

Base substitutions
A type of mutation in which one base is replaced by another in DNA.

Insertions and deletions
(Indels). A type of mutation that arises from the insertion or deletion of one or more nucleotides within a DNA sequence.

Structural variations
Large-scale genomic changes (typically > 1 kb) such as deletions, tandem duplications, amplifications, inversions and translocations.

Transversions
Mutations that involve different classes of nucleotides; that is, purine-to-pyrimidine or pyrimidine-to-purine mutations.

Transitions
Mutations that involve the same class of nucleotides; that is, purine-to-purine or pyrimidine-to-pyrimidine mutations.

could be used as prognostic indicators, as predictors of therapeutic sensitivity or as targets of disease control.

In this Review, we present examples of mutational signatures according to different classes of mutations, including base substitutions, insertions and deletions (indels), and structural variations (also known as genomic rearrangements). We emphasize how different DNA damaging agents and DNA repair and replication pathways contribute mechanistically in the generation of each signature type, and our main purpose is to show the wealth of biology that could be discovered in the totality of somatic mutations.

Mutational signatures of base substitutions

Historically, simple analyses of somatic base substitutions as six-bar mutational spectra (C·G→A·T, C·G→G·C, C·G→T·A, T·A→A·T, T·A→C·G and T·A→G·C) have been useful in highlighting typical but crude mutation patterns that show how mutational spectra can be specific to tumour type and related to exogenous carcinogens. For example, mutations associated with smoking-related damage in lung cancers are mainly G·C→T·A transversions[11], whereas mutations associated with ultraviolet (UV) radiation exposure in skin cancers comprise predominantly C·G→T·A transitions[8]. However, the flanking sequence context of a mutation (that is, the neighbouring bases immediately 5′ and 3′ to the mutated base) is known to affect mutation rates in the genome[12] and should therefore be taken into consideration when defining a mutational signature. As there are 6 classes of base substitutions and 16 possible sequence contexts for each mutated base (A, C, G or T at the 5′ base and A, C, G or T at the 3′ base), 96 different mutated trinucleotides are possible[2,9,10]. The following convention has been adopted to describe mutations; for example, a cytosine mutation flanked by a 5′ thymine and a 3′ guanine is represented as TpCpG, and the mutated base is underlined.

In a recent mathematical analysis, 21 different mutational signatures were identified in 96-trinucleotide format from the somatic mutations of >7,000 sequenced primary human cancers of 30 different cancer types[9]. Although some of these signatures were known (for example, an excess of C·G→T·A transitions particularly at dipyrimidines (Signature 7) has previously been shown to be associated with UV radiation and is found in cutaneous malignancies[13]), many were novel. Importantly,

Box 1 | **Extracting mutational signatures from complex data sets**

Exploring the sequence context of somatic substitutions in cancer

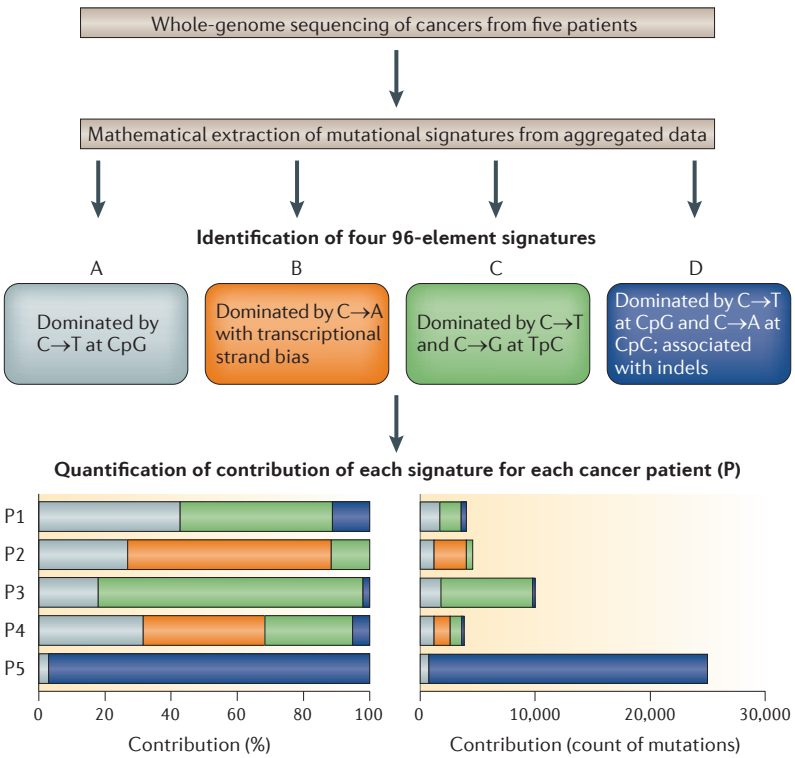
Mathematical approaches can be used to identify mutational patterns in a data set of base substitutions pooled from several cancer samples obtained from multiple patients (see the figure). The process of extracting multiple patterns from a complex multidimensional pool of data is described as a blind source separation problem. In this case, the multidimensional data set comprises the 96 possible combinations of base pair substitution mutations (that is, when the immediate flanking sequence context is taken into account). Adding an additional flanking base to the one immediately adjacent to the mutation would give 1,536 possible mutations, and the number of mutations per cancer genome will then become the statistically limiting factor. Several different mathematical methods can be applied to solve this problem.

A mathematical approach for extracting mutational signatures

Non-negative matrix factorization (NMF) and model selection is simply one of many approaches that have previously been developed to factorize or reduce complex multidimensional data sets in order to identify common, defining underlying patterns that make up a pooled data set[113]. Consider that each 96-element data set is akin to a 'face' of a cancer. Each of these cancer faces is similar to a human face and is a complex assembly of features; nevertheless, it is recognizable as an individual face. The application of NMF to a pool of images of faces yields interpretable underlying 'features' that are shared across the group of faces, such as the eyes, nose and mouth. The aggregate of somatic substitutions of each cancer is essentially the face of a cancer, and each extracted feature is equivalent to an individual mutational signature. In this case, aggregated data are parsed through NMF in order to obtain the signatures that underlie the data sets, and 96-element signatures are extracted (see the figure, Signatures A–D).

Quantifying the amount of each signature in each cancer

For each mutational signature, NMF allows estimation of the relative contribution of each signature to the final mutational catalogue of individual cancers (see the figure). The amount of each signature can be quantified in each cancer either as a proportional contribution or as absolute numbers. NMF can therefore both highlight cancers that are driven predominantly by a single mutational signature and identify cancers that have a combination of many different signatures (see the figure). NMF can identify even the lowest levels of signatures that are ubiquitously present[9].



each base substitution signature represents a pattern that consists of 96 elements, which vary in their relative amounts. A particular element — such as C·G→T·A transitions at TpCpN (where N denotes any base) — may be the overriding feature within a mutational signature, but the element is not considered to be a signature per se.

Below, we consider some examples of base substitution signatures on the basis of the different categories of mutational processes that underlie each signature. Mechanistically, each mutational process comprises both a DNA damage component and a DNA repair or replicative component (FIG. 2). Each type of DNA damage has its own predilection for specific nucleotides, which can produce recognizable patterns of mutagenesis. The most prominent base substitution signatures are illustrated (FIG. 2) to show the 96-element pattern of each signature, as well as the DNA damage and repair or replication components that constitute the determinant mutational process.

Mechanisms underlying substitution signatures

Endogenous DNA damage. Several different 96-element mutational signatures have been linked to mutagenic processes that are attributed to deamination, which occurs spontaneously in all DNA bases that contain primary amines albeit at markedly different rates. Common deamination reactions include 5-methylcytosine→thymine, cytosine→uracil and adenine→hypoxanthine reactions.

The hydrolytic deamination of 5-methylcytosines at CpG dinucleotides[14] has occurred so frequently throughout evolution that it is thought to be the reason for the depletion of the number of methylated CpGs observed in the human genome[14]. Despite the reduction in absolute numbers of these sites, it remains one of the most mutagenic sequence motifs, and a net effect of C·G→T·A transitions is observed at methylated CpG dinucleotides. Consistent with this phenomenon, C·G→T·A substitutions at NpCpG are characteristic of two of the most frequent mutational signatures — Signatures 1A and 1B — which have collectively been documented in at least 25 different cancer types[9]. These signatures possibly represent one biological process but tend to be separated mathematically because of limitations regarding the number of samples in the data sets examined so far and the algorithm used[9,15]. Intriguingly, a correlation between the burden of mutations associated with these signatures and the patient age at the time of cancer diagnosis has been reported for several cancer types, including adult cancers (for example, acute myeloid leukaemia, breast cancer, glioma, head and neck cancers, kidney clear cell cancer, malignant melanoma and ovarian cancer) and paediatric cancers (for example, acute lymphoblastic leukaemia and neuroblastoma) in both males and females[9]. This suggests that this mutational process is occurring in cells prior to malignant transformation.

The deamination process of cytosine to uracil is thought to be catalysed by members of the cytidine deaminase family (which include activation-induced cytidine deaminase (AICDA) and the APOBEC (apolipoprotein B mRNA editing enzyme, catalytic

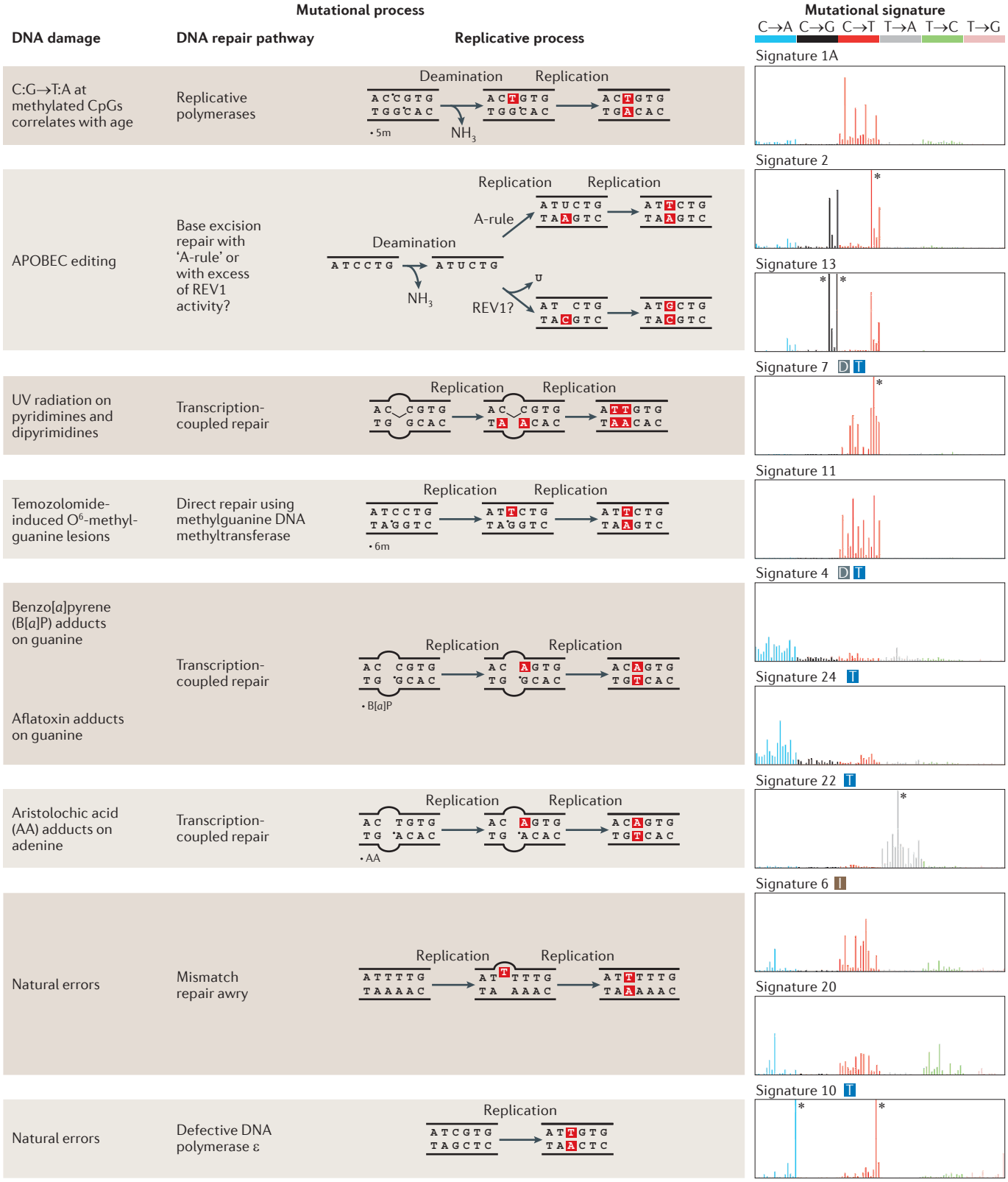
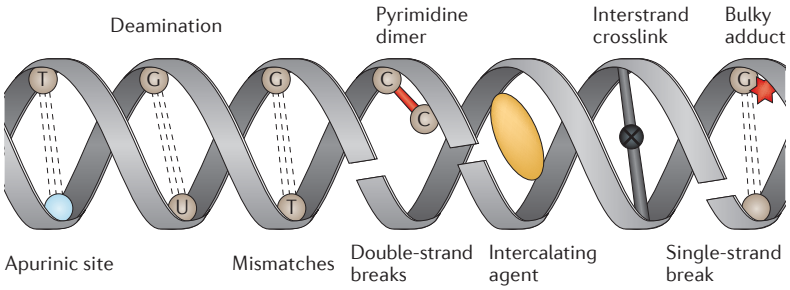


Figure 2 | **Summary of known mutational signatures, and the components of DNA damage and repair that constitute the mutational processes.** There are marked differences among the 96-element mutational signatures, which are dominated by specific elements, including enrichment of various base substitutions (shown in the graphs on the right), transcriptional strand bias (T), excess of dinucleotide mutations (D), and association with insertions and deletions (I). The asterisks mark instances at which the limits of the *y* axes, which represent the likelihood of specific mutations being present in a signature, are exceeded. 5m, 5′ methyl group; 6m, O^6 methyl group; APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide; REV1, DNA repair protein REV1; UV, ultraviolet.

Box 2 | **An overview of types of DNA damage and causal agents**



DNA is under a constant stream of attack from various exogenous and endogenous sources. Each mutagen can cause damage either directly or indirectly to the nucleotides in the genome. Moreover, each mutagenic agent shows a predilection for damaging specific nucleotides, which can produce recognizable patterns of mutagenesis.

Sources of DNA damage include endogenous factors such as spontaneous or enzymatic conversions. The *N*-glycosidic bond that links a nucleobase and a pentose sugar to form a nucleoside is labile. This fact underlies the common occurrence of spontaneous base loss in DNA (~10^4 bases per cell per day)[27], which results in the formation of apurinic or apyrimidinic sites (see the figure). Depurination occurs more readily than depyrimidination, which makes apurinic sites more common than apyrimidinic sites, and A·T→T·A or G·C→T·A transversions arise depending on the purine that is lost.

Other types of endogenous DNA damage include deamination, replication errors and free radical species. Free radical species are generated either as a by-product of metabolism or through exposure to exogenous physical agents, such as ionizing radiation, which can induce the formation of double-strand breaks. By contrast, non-ionizing ultraviolet radiation is responsible for biochemical modifications, such as the formation of pyrimidine dimers, which can be mutagenic when left unrepaired. Other external agents that are known to cause DNA damage include chemical compounds, for example, platinum-based compounds such as cisplatin, which can cause bulky adducts or interstrand and intrastrand crosslinks; intercalating agents such as benzo[*a*]pyrenes, daunorubicin and actinomycin-D; DNA alkylating agents such as nitrogen mustards, methyl methanesulphonate (MMS), *N*-nitroso-*N*-methylurea (NMU) and *N*-ethyl-*N*-nitrosourea (ENU); and psoralens.

polypeptide) enzymes). AICDA is the most well characterized of this family of DNA editing enzymes; it has a role in antibody diversification and shows a strong preference for deaminating cytosine residues that are flanked by a 5′ purine[16]. By contrast, the APOBECs — which have variable roles, including restriction of retroviruses and mobile retroelements — show various sequence specificities, for example, APOBEC1, APOBEC3A, APOBEC3B and APOBEC3C show a preference for a TpC sequence context in experimental systems such as yeast and human cell lines[17–19]. First characterized in breast cancers[2,5,20], signatures with a thymine preceding a mutated cytosine (TpCpN; Signatures 2 and 13) have been observed in 16 other cancer types[9]. Particular members of the cytidine deaminase family (APOBEC3A, APOBEC3B and APOBEC1) have been speculated to underlie this phenomenon given the similarity between sequence specificity observed in cancers and that observed *in vitro*[2,19]. Aggregated expression-based analyses have shown correlations with the burden on mutated cytosines at a TpCpN context, which led the authors to suggest that APOBECs are a mutagenic source of these signatures[21,22]. There is additional support for a role of APOBECs in the generation of the DNA damage component of these signatures. Intriguingly, mutations associated with Signatures 2 and 13 show a high degree of strand coordination: they arise on the same parental allele and are on the same DNA strand; that is, successive mutations can be C→T then C→G followed by C→T, or G→A then G→C followed by G→A, but not C→T, G→A followed by C→T)[2,9,23]. This strand-coordinated nature of TpCpN mutations argues in favour of APOBEC-related activity, as APOBECs preferentially cause deamination of stretches of single-stranded DNA (ssDNA)[23–25]. Furthermore, a germline copy-number polymorphism involving the neighbouring *APOBEC3A* and *APOBEC3B* genes that essentially deletes all of the genomic region encompassing *APOBEC3B* apart from its 3′ untranslated region has been shown to act as a modest susceptibility allele in breast cancer[23]. Carriers of at least 1 copy of the deletion polymorphism have a 2.37-fold increased relative risk of harbouring cancers that comprise Signatures 2 and 13. Interestingly, although these two signatures are likely to arise through the same DNA damage mechanism of APOBECs, Signature 13 is dominated by C·G→G·C transversions. In other words, the sequence context of mutated cytosine bases is shared with Signature 2 (TpCpN) because the DNA damaging enzyme is possibly identical; however, the excess of transversions in Signature 13 relative to transitions in Signature 2 suggests subtly different involvement of repair or replicative polymerases (see below) between the two signatures (FIG. 2).

Adenine can deaminate to hypoxanthine at a rate of 10% of the cytosine deamination rate[26]. The product pairs preferentially with cytosine during replication and can give rise to A·T→G·C transitions[27]. Several signatures characterized by A·T→G·C transitions (Signatures 5, 12, 16 and 21) have been found in primary human cancers, although none has been specifically attributed to this mutational process so far.

Free radical species such as reactive oxygen species or nitrogen oxide species are generated endogenously as by-products of normal cellular metabolism, including apoptosis and the inflammatory response, as well as by exposure to exogenous agents such as ionizing radiation[28]. Their interaction with DNA can lead to >25 different oxidative DNA base lesions[29]. One of the best studied oxidative DNA lesions of reactive oxygen species is 8-oxo-2′-deoxyguanosine. It has been shown to favour hydrogen bonding with adenine, which gives rise to G·C→T·A transversions with evidence for GpGpG sequence specificity *in vitro*[30,31]. A mutational signature derived from primary human cancers has not been attributed to this oxidative DNA lesion, although two novel signatures are noted to mainly comprise G·C→T·A mutations (Signatures 8 and 18)[9].

Exogenous DNA damage. Environmental sources of DNA damage can be physical or chemical (BOX 2). Non-ionizing UV radiation is an example of a physical agent with enough energy to excite molecular bonds that cause covalent modifications between neighbouring pyrimidine nucleotides. These modifications result in pyrimidine dimers: (6–4) pyrimidine photoproducts ((6–4)PPs) and cyclobutane pyrimidine dimers

Deamination
A biochemical reaction that removes an amine group from a molecule.

(CPDs)[13,32]. Consistent with this finding, a preponderance of C·G→T·A mutations at dipyrimidines (that is, two adjacent pyrimidines) and an excess of CC·GG→TT·AA double substitutions (Signature 7) (FIG. 2) are characteristic features of cutaneous cancers that are associated with UV exposure, such as squamous cell skin carcinomas and malignant melanomas[9,33]. Indeed, the effect is so pronounced that CC·GG→TT·AA double substitutions can constitute up to 25% of the total (and often very large) mutation burden in those cancers[9] and can be used as a clear indicator of UV-related DNA damage. Mechanistically, Signature 7 is caused by deamination of cytosines to uracil within (6–4)PPs or CPDs at sites of stalled transcription complexes[33], which triggers the activity of transcription-coupled repair (TCR; see below). This process explains why the signature shows a transcriptional strand bias[9] (that is, a lower prevalence of mutations on the transcribed strand than on the non-transcribed strand).

Chemical compounds intercalate or covalently bind to DNA in various ways and can produce particular mutational signatures. For example, chemotherapeutic alkylating agents such as cyclophosphamide and temozolomide result in C·G→T·A transitions[5] (Signature 11)[9], whereas benzo[*a*]pyrene (B[*a*]P) diol epoxides — a carcinogenic by-product of tobacco smoking[34,35] — cause G·C→T·A transversions and have a predilection for methylated CpG dinucleotides[11] (Signature 4)[9] (FIG. 2). Psoralens, which are a type of phototherapeutic agent used for inflammatory conditions such as psoriasis, lead to pyrimidine mutations at a TpA sequence context[36,37]; aristolochic acid, which is a plant extract linked to nephropathy and urothelial tumours, is associated with a T·A→A·T signature[38] (Signature 22) (FIG. 2). These examples highlight the variability in mutational signatures that can be produced through a myriad of exposure to chemicals. The ability to pinpoint chemical mutagens to specific signatures means that a patient's history of past exposure to specific chemicals could be revealed by analysing their tumours. Many other chemical compounds are known to cause DNA damage (BOX 2), although specific signatures remain to be discovered and assigned to these agents.

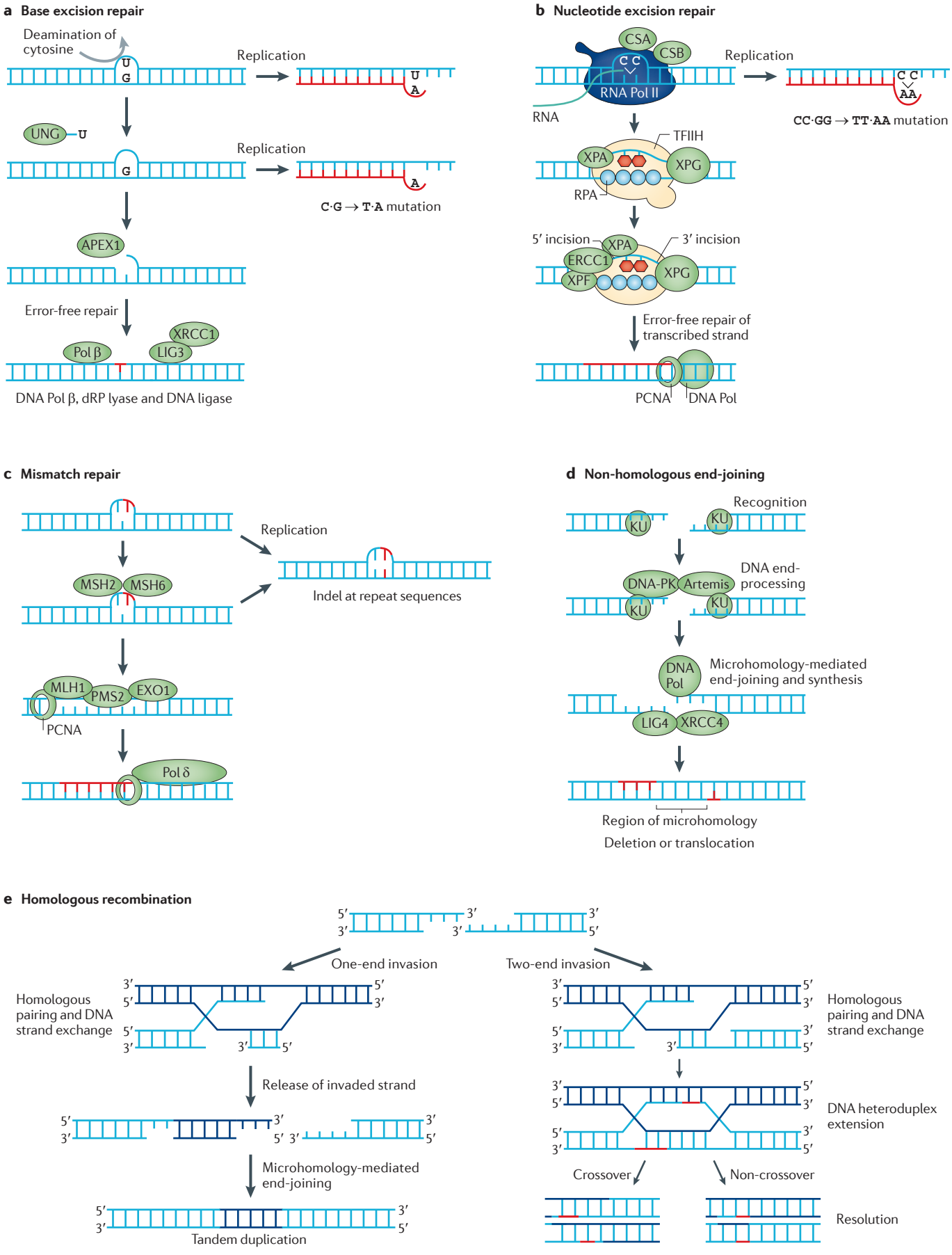
DNA repair processes. It is impossible to exhaustively describe all repair pathways here; hence, we give brief descriptions that focus on how each repair pathway leaves its molecular mark on a genome and how its disruption can result in specific mutational signatures (FIG. 3).

In base excision repair (BER), a base lesion is identified by a DNA glycosylase that recognizes, hydrolytically cleaves and removes the altered base, which gives rise to an apurinic or apyrimidinic site[39] (FIG. 3a). Unrepaired apurinic or apyrimidinic sites are particularly mutagenic, as incorrect bases are easily introduced during replication. Subsequently, DNA-(apurinic or apyrimidinic site) lyase APEX1 incises the DNA strand 5′ to the apurinic or apyrimidinic site. The replicative DNA polymerase β (Pol β) catalyses the elimination of the 5′-deoxyribosophosphate residue and then fills the one-nucleotide gap.

Figure 3 | **DNA repair pathways and mutational consequences.** **a** | Base excision repair (BER) typically mediates the removal and replacement of a single base residue. Substrates include uracil residues in DNA (which are created by deamination of cytosines) and damaged bases caused by reactive oxygen species, hydrolytic reactions and methylation. A damaged base is removed by a specific DNA glycosylase; here, the uracil is removed by uracil-DNA glycosylase (UNG). The resulting apurinic or apyrimidinic site is incised by DNA-(apurinic or apyrimidinic site) lyase APEX1. The 5′-deoxyribose-phosphate (dRP) residue is removed by a dRP lyase, which leaves a one-nucleotide gap that is filled in by DNA polymerase β (Pol β). Replication before completion of repair leads to base misinsertion and potentially C·G→T·A mutations. **b** | Nucleotide excision repair (NER) can remove various helix-distorting adducts, including those caused by ultraviolet radiation and cisplatin. The distorted region is recognized either during global genome repair by XPC (DNA repair protein complementing XP-C cells)–RAD23B (not shown) or during transcription, and two incisions are made on either side of the adduct to excise the damaged DNA. The resulting 27–29-nucleotide gap is filled by Pol δ or Pol ε and, under some circumstances, Pol κ. Replication before repair may result in mutations. **c** | Mismatch repair (MMR) is an excision repair process that removes mismatched bases or misinserted bases in DNA. It is initiated by the DNA mismatch recognition proteins MSH2 and MSH6; a segment of DNA is excised between the mismatch and a nearby nick by the MMR endonuclease PMS2 and exonuclease 1 (EXO1). The gap that is left in the DNA is filled by Pol δ. Failed MMR results in a high mutation load in microsatellite repeat sequences. **d** | DNA double-strand breaks (DSBs) can be repaired by non-homologous end-joining (NHEJ), which is often mediated by microhomology at ends. DSBs caused by ionizing radiation or by enzymes that cleave DNA usually do not yield DNA ends that can be ligated directly. End-trimming and resynthesis of bases are therefore required to join breaks, which may give rise to mutations. **e** | An alternative strategy for DSB repair is homologous recombination (HR). HR only operates when a double-stranded copy of the sequence is available, for example, as a sister chromatid in late S or G2 phase of the cell cycle, which may give rise to tandem duplication. CSA and CSB are also known as DNA excision repair protein ERCC8 and ERCC6, respectively; DNA-PK, DNA-dependent protein kinase; indel, insertion and deletion; LIG3, DNA ligase 3; PCNA, proliferating cell nuclear antigen. Figure from REF. 114, Nature Publishing Group.

Finally, the nick is sealed by the DNA ligase III–XRCC1 complex[40,41] (FIG. 3a). Multiple mutation patterns have been associated with engineered defects of certain DNA glycosylases in mouse embryonic fibroblasts. For example, defects in single-strand selective monofunctional uracil DNA glycosylase (SMUG1) have been linked to C·G→T·A transitions[42], whereas disruption of the DNA glycosylase OGG1 has been associated with G·C→T·A transversions[43]. However, 96-element signatures extracted from human cancers have not been attributed to defects in specific components of the BER pathway so far.

Transcriptional strand bias
Bias in mutation load between the transcribed strand and the non-transcribed strand.



Nucleotide excision repair (NER) is a nonspecific repair process that is activated upon sensing of bulky DNA distortions, for example, bulky adducts caused by B[*a*]Ps and by aromatic amines such as aflatoxin, as well as modifications due to platinum-based compounds, psoralens and UV-induced lesions (that is, CPDs and (6–4)PPs) (reviewed in REF. 44) (FIG. 3b). A particular class of NER that is coupled to transcription is TCR[44]. A consequence of TCR is that DNA damage on the transcribed strand is repaired more efficiently than that on the non-transcribed strand. The activity of TCR is appreciable in several mutational signatures. For example, C·G→T·A transitions that constitute the UV-associated Signature 7 show transcriptional strand bias; that is, fewer mutations are found on the transcribed strand than on the non-transcribed strand[45]. This bias is also seen in other mutational signatures, including those caused by B[*a*]Ps[34] (Signature 4) and aristolochic acid[46] (Signature 22). Several novel signatures that show transcriptional strand bias (Signatures 5, 8, 12 and 16) have additionally been identified[9], which suggests that these could be caused by DNA damaging agents that are repaired by TCR. However, BER was recently shown to also display transcriptional strand bias[47], which suggests that there are alternative mechanisms that can generate strand bias in these signatures.

The post-replicative mismatch repair (MMR) system recognizes and repairs misincorporated bases, as well as erroneous indels that arise during DNA replication and DNA recombination repair activity (extensively reviewed in REFS 48,49) (FIG. 3c). MMR reduces the rate of replication-associated errors by 100-fold to 1 in 10^{-9} (reviewed in REF. 48). Hence, defects in the MMR pathway increase the spontaneous mutation rate[50]. Mutations in MMR-related proteins affect genomic stability and result in microsatellite instability[51]. MMR-related base substitution signatures have not been previously shown in experimental systems. Nevertheless, a base substitution signature extracted from primary human cancers (Signature 6) — which is characterized by C·G→T·A transitions at an NpCpG sequence context and C·G→A·T transversions at CpCpC — has been associated with MMR deficiency (biallelic somatic mutations in MMR genes and particularly those affecting MLH1 methylation)[9]. Furthermore, cancers that contained a high proportion of this signature also showed thousands of small 1-bp indels, which is a feature associated with microsatellite instability[9]. More recently, additional signatures (Signature 20 and a new pattern, Signature 26) have been additionally associated with MMR deficiency (S.N.-Z., unpublished observations). These may relate to specific MMR defects, although the data required to confirm this are not currently available.

DNA replication errors. Given the size of the human genome ($\sim 3 \times 10^9$ nucleotides), even the smallest error rate during DNA synthesis can result in many mutations, which underscores the replication machinery as a source of mutagenesis. DNA polymerases use a template DNA strand to select nucleotides for incorporation into the nascent strand during both DNA replication and synthesis associated with DNA repair; however, replication mismatches can be generated on the nascent strand (reviewed in REF. 52). The high-fidelity B family DNA polymerases Pol δ and Pol ε have an error rate of 1 in 10^{-7} for every nucleotide synthesized owing to intrinsic proofreading properties[53]. Somatic and germline mutations in Pol ε have been associated with Signature 10 in colorectal and endometrial carcinomas[9,54,55], and they result in a striking pattern of C·G→A·T and C·G→T·A mutations at TpCpG (FIG. 2). It has been suggested that the increased rate of mutagenesis associated with mutations in Pol ε exceeds that expected after loss of proofreading capacity, which indicates a distinct defect of replication fidelity or an active mutagenic process[56].

An additional factor that affects the likelihood of nucleotide misincorporation by replicative DNA polymerases such as Pol δ and Pol ε is the balance of the cellular deoxynucleoside triphosphate (dNTP) pool. Loss of the usual constraints on cell cycle regulation during cancer development causes an increased demand on a potentially reduced dNTP pool. Perturbations of the dNTP pool can lead to insertion–deletion loops and erroneous base incorporation; they can also affect proofreading efficiency[57] and be another source of replication-related mutagenesis[58–60].

A collection of low-fidelity error-prone polymerases — such as Pol η, Pol ι, Pol κ and DNA repair protein REV1 — can replicate damaged DNA or non-informative DNA templates. These translesion polymerases have a higher error rate (which ranges between 1 in 10^{-4} and 1 in 10^{-1}) than nuclear DNA replication polymerases because they lack proofreading capacity and are poor discriminators of mismatched, non-fitting nucleotides (reviewed in REF. 61). This phenomenon known as DNA damage tolerance (reviewed in REFS 61–63) is crucial to allow completion of replication at the cost of introducing errors — which may be fixed later by excision repair pathways — and to avoid replication fork collapse (FIG. 4). However, by providing this escape route, translesion polymerases can produce a myriad of potential mutational spectra. For example, the preference for insertion of an adenine opposite an apurinic or apyrimidinic site (that is, the 'A-rule')[64] results in different signatures depending on the original base at the site: adenine loss would lead to A·T→T·A, whereas guanine loss would lead to C·G→A·T. A signature characterized by T·A→G·C transversions at ApTpN and TpTpN trinucleotides (Signature 9), which is seen in haematological malignancies that have undergone somatic hypermutation at the immunoglobulin (*IG*) loci, has been attributed to the activity of Pol η[65], although the precise mechanism remains unclear. Moreover, the error-prone polymerase REV1 generates a C·G→G·C signature[61,66] (FIG. 2).

Taken together, these studies indicate that irrespective of the damaged base, the resulting mutation — regardless of whether it is a transition or transversion — is likely to be determined by the replicative process.

Mutational signatures of indels

Indel signatures. Modern mathematical methods for extracting base substitution signatures can be integrated

Microsatellite instability
Variability in the length of base pair repeated sequences (< 5 bp) that is caused by replication slippage and that is normally kept stable by mismatch repair.

Replication fork collapse
A condition at a replication fork in which the integrity of a DNA molecule is impaired and can result in a DNA double-strand break.

Somatic hypermutation
Regional hypermutation at the immunoglobulin locus that generates antibody diversity.

with other mutation classes (such as indels) to provide important insights. Although indels can be of any size, we focus on small indels (<100 bp) here. Currently, calling somatic indels from next-generation short-read sequencing data still yields a high rate of false positives owing to technical difficulties associated with mapping of short-read sequencing data and to limitations of mutation-calling algorithms. Additionally, as fewer indels are generally identified in human cancers than base substitutions[9], the power to detect patterns of indel generation is relatively limited. Nevertheless, some early patterns can be derived from analyses based on the size of indels and on the characteristics of the deletion junctions.

Small 1–3-bp indels within repetitive sequences correlate with a base substitution signature that is characterized by an excess of C·G→T·A mutations at NpCpG (Signature 6)[9]. Individual cancers can be overloaded both by mutations associated with Signature 6 and by small indels, as has been reported in colorectal, uterine, kidney, liver, prostate, oesophageal and pancreatic cancers. By contrast, larger indels (between 4 bp and ~50 bp) that show a degree of sequence similarity between the indel motif and the immediate junction sequence (that is, microhomology) have been associated with a base substitution signature that is characterized by a fairly uniform distribution of mutations across all 96 possible base substitution types (Signature 3)[9]. This signature has been reported in breast, ovarian and pancreatic cancers[9].

Mechanisms of indel signature formation. The two contrasting indel signatures described above are thought to arise as a result of defects in the DNA repair machinery. For example, loss of MMR in humans leads to microsatellite instability — an indel phenomenon that can be recognized owing to variation in repeat length at mononucleotide or dinucleotide repetitive sequences, which is frequently observed in colorectal carcinomas[67,68]. The mechanistic importance of post-replicative MMR as a constraint on the generation of indels during replication is emphasized by studies showing that spontaneous indel error rates in repetitive sequences increase by many orders of magnitude when MMR is inactivated, and this is shown to be an overwhelming feature of cancers of individuals with inherited germline mutations in MMR genes[69,70]. Consistent with these reports, the excess of Signature 6 and the associated abundance of small indels (1–3 bp) at polynucleotide tracts are concomitant with inactivation of the MMR genes in affected cancers[9]. In addition, a signature of indels on a background of MMR deficiency is highly reproducible in experimental systems[71].

Overlapping microhomology is often considered to be a signature of non-homologous end-joining (NHEJ) repair of DNA double-strand breaks (DSBs), in which short segments of homology are aligned to mediate the joining of the two DNA fragments[72,73] (FIG. 3d). Signature 3 is associated with inactivating mutations of *BRCA1* (breast cancer 1, early onset) and *BRCA2* (REF. 9). The protein products of *BRCA1* and *BRCA2* are involved in error-free homologous recombination-based DSB repair[74,75], in which BRCA1 controls resection of DNA ends[76] and BRCA2 is required for loading of RAD51 onto ssDNA[77]. Thus, the increased frequency of microhomology-mediated indels in *BRCA1*- or *BRCA2*-null cancers might reflect the requirement for alternative methods of DSB repair in these cancers. However, it

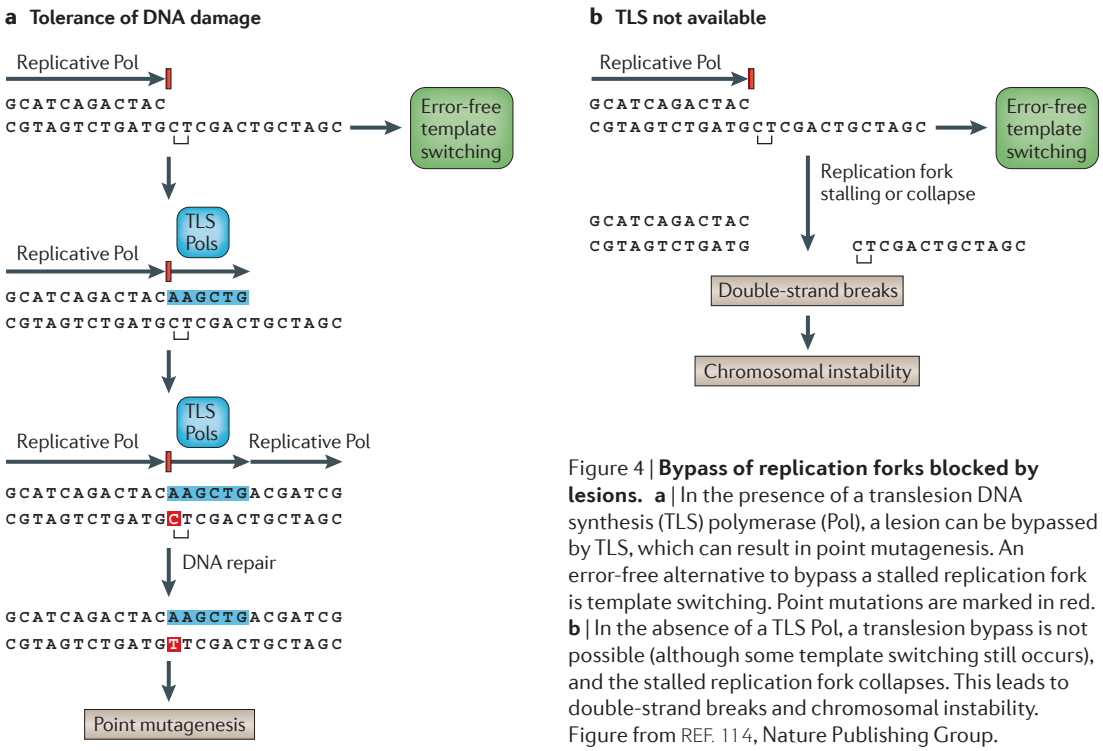


Figure 4 | **Bypass of replication forks blocked by lesions.** **a** | In the presence of a translesion DNA synthesis (TLS) polymerase (Pol), a lesion can be bypassed by TLS, which can result in point mutagenesis. An error-free alternative to bypass a stalled replication fork is template switching. Point mutations are marked in red. **b** | In the absence of a TLS Pol, a translesion bypass is not possible (although some template switching still occurs), and the stalled replication fork collapses. This leads to double-strand breaks and chromosomal instability. Figure from REF. 114, Nature Publishing Group.

remains unclear how defects in these two distinct components of the homologous recombination pathway can result in a final characteristic readout of a somatic base substitution that correlates with a signature of larger, microhomology-mediated indels (Signature 3). This signature may either reflect the supplementary roles of BRCA1 or BRCA2 in the response to DNA damage or be a result of the increased recruitment of error-prone polymerases to compensate for the inability to use homologous recombination to bypass a lesion.

Mutational signatures of structural variations

The landscape of somatically acquired rearrangements is extremely diverse and ranges from very few mutations to tens or hundreds of mutations per cancer[78]. Some cancer-associated rearrangements are functional driver events and are under strong selection, including amplification of oncogenic regions, whole-exon or whole-gene deletions, losses of whole chromosomal arms that involve tumour suppressor genes and translocations that produce oncogenic fusion genes[79]. However, most rearrangements are passenger events[78]. The ability to call somatic rearrangements from next-generation sequencing data is still fraught with suboptimal sensitivity and specificity owing to the limitations of current rearrangement-calling algorithms. Hence, cancer genome data sets are not as comprehensively characterized for structural variations as they are for base substitution mutations. Nevertheless, the patterns of somatic rearrangements, their spatial distribution throughout the genome and the junctional features at breakpoints of available rearrangement data sets reveal some mechanisms of damage and repair that are involved in the generation of somatic structural variations.

Structural variations arise from DSBs through either direct or indirect mechanisms, which can determine the resulting molecular signature. Primary DSBs are due to direct lesions that cause breaks in the sugar-phosphate backbone (for example, by ionizing radiation), whereas secondary DSBs are the result of complex DNA lesions which, when encountered by a replication fork, induce replication collapse[80,81]. Each type of DSB repair mechanism will leave its own characteristic imprint of activity in the genome.

Microhomology-mediated end-joining (MMEJ) is a subtype of NHEJ (FIG. 3d), in which the ligation is facilitated by microhomologies between ssDNA exposed at the DNA ends as a result of limited end-processing activities. MMEJ is commonly involved in somatic structural variation from primary cancers and cell lines[78], as well as from experimental DSB repair models[72], particularly in systems in which homologous recombination is defective. In mammalian somatic cells, NHEJ and MMEJ activity on double-ended DSBs occurs throughout the cell cycle[82], whereas homologous recombination acts on replication-associated or G2-induced double-ended DSBs at which a homologous sister chromatid is available[83,84]. The near-constant action of NHEJ throughout the cell cycle makes its contribution almost ubiquitous in all forms of structural variations. The mark of NHEJ is essentially the absence of sequence homology or, more commonly, the presence of MMEJ at breakpoints in a distribution that is different to that expected if microhomology had occurred randomly[78,85]. Unsurprisingly, MMEJ of all forms of structural variations has been reported[86–88].

Tandem duplications. Rearrangements of tandem duplications (that is, identical sequences duplicated in head-to-tail formation) with microhomology junctions have been reported in breast[2,78] and ovarian cancers[89]. Some of these cancers have shown biallelic loss of *BRCA1* (REFS 2,78). Interestingly, a specific homologous recombination subpathway that is distinct from RAD51-mediated homologous recombination has been implicated in the generation of tandem duplications[90]. In this pathway, DNA ends at DSBs that occur at replication forks invade the sister chromatid to restart replication in a process known as break-induced replication (BIR)[91] (FIG. 5a). The invaded strand can be released by branch migration and the new extended double-stranded DNA end repaired by MMEJ, which leaves a tandem duplication[90]. This combination — termed synthesis-dependent end-joining (SDEJ) — has previously been described to provide an explanation for tandem duplications in mammalian genomes[92]. Specifically, SDEJ is initiated in a similar manner to all homologous recombination events by resection of the DNA end, followed by strand invasion of the sister chromatid and DNA extension on the D-loop[93] (FIG. 3e; FIG. 5a). However, unlike the synthesis-dependent strand annealing model for DSB repair[94], synthesis on the lagging strand is also initiated on the sister chromatid, and the released DNA molecule will be partly double-stranded. When this is ligated onto the opposite DNA end (using MMEJ), a tandem duplication is produced as replication extends beyond the original breakpoint (FIG. 5a). Upsetting the balance of error-free homologous recombination-based DSB repair could result in upregulation of other components of DSB repair, such as SDEJ, and result in the tandem duplication signature. This possibility reflects the complex nature of homologous recombination, which involves several pathways with specific enzymatic requirements.

Clustered structural variations. Somatic structural variations — for example, oncogenic amplifications such as *HER2* (also known as *ERBB2*) in breast cancer — are regional or topographically clustered[95]. These somatic events show high levels of copy number (>5) and many types of microscopic rearrangements within a macroscopic region. They are also recurrent (as a result of positive selection) and show concomitant elevated levels of expression of the relevant oncogene[95]. The exact mechanisms that cause gene amplification in cancer remain unclear. The model originally proposed by Barbara McClintock in 1938 suggests that intrachromosomal cycles of breakage–fusion–bridge initiated by a DSB can promote progressive acquisition of additional genomic alterations that result in localized amplification[96,97] (FIG. 5b). If this hypothesis is true,

Synthesis-dependent end-joining
(SDEJ). A process in which a DNA end at a double-strand break is extended using the intact sister chromatid as template. The DNA end is released from the sister chromatid and rejoined by end-joining.

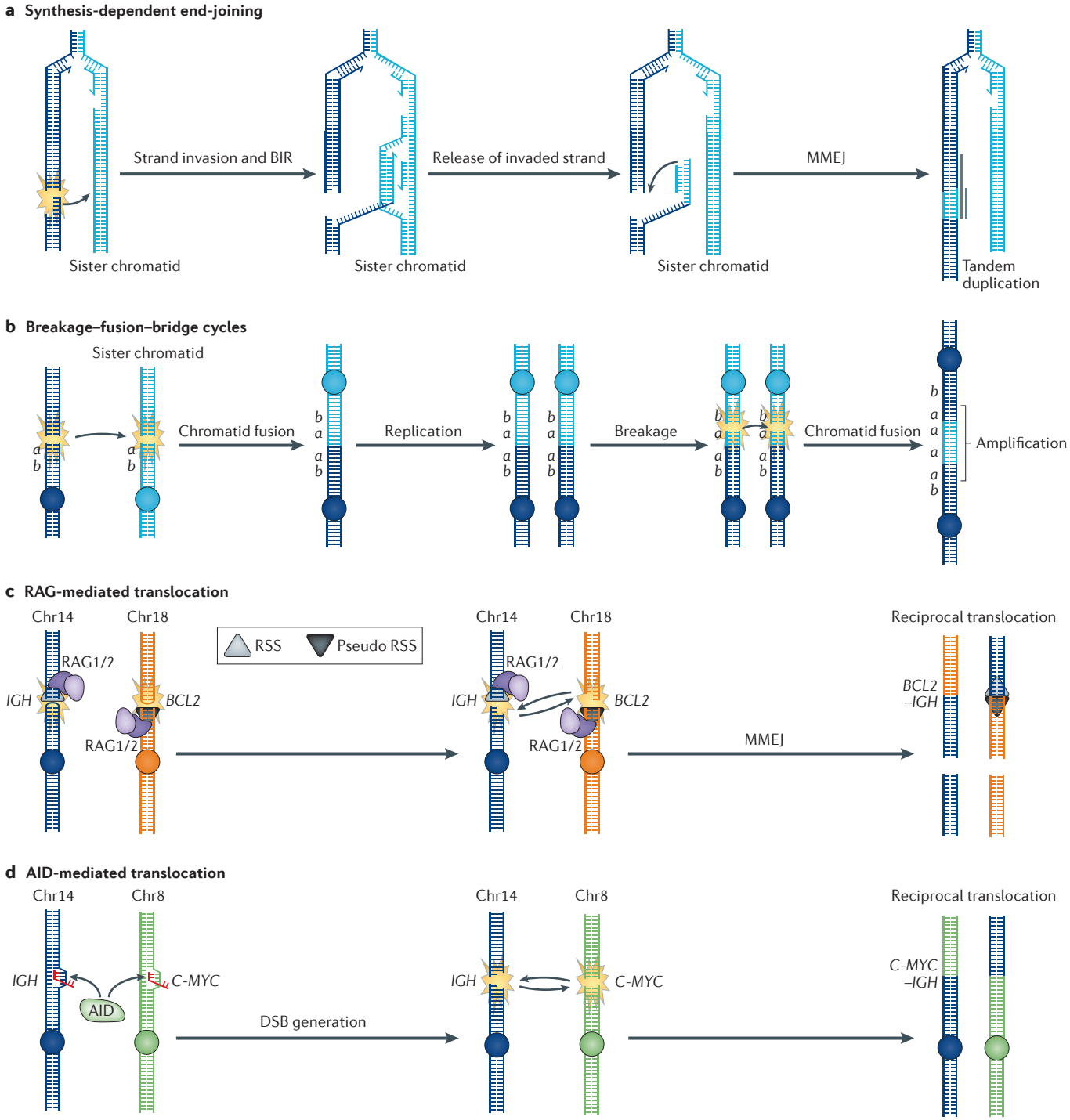


Figure 5 | **Gene rearrangements in cancer.** Gene rearrangements in cancer arise primarily from DNA double-strand breaks (DSBs). **a** | Synthesis-dependent end-joining (SDEJ), which is involved in repairing replication-associated DSBs, results in tandem duplications. Break-induced replication (BIR) initiates synthesis on the sister chromatid after strand invasion. Reversed branch migration of the Holliday junction formed following strand invasion can release the invaded strand, which contains extra DNA material from the sister chromatid and is fused to the original end by microhomology-mediated end-joining (MMEJ), resulting in a tandem duplication[90]. **b** | Sister chromatid fusion causes gene amplification by breakage–fusion–bridge cycles[96,97]. In this process, two adjacent DSBs on sister chromatids are substrates for non-homologous end-joining (NHEJ), which rejoins the sister chromatids. After replication, these are again broken to form anther fusion chromosome carrying four gene *a* copies. **c** | The V(D)J recombination-activating (RAG) proteins recognize either the correct recombination signal sequences (RSSs) or almost identical (that is, pseudo) RSSs at which they initiate DSBs; they then mediate interchromosomal translocation rather than regular recombination within the V(D)J segments. This can create an immunoglobulin H (*IGH*)–*BCL2* (B-cell CLL/lymphoma 2) fusion gene that drives cancer. **d** | Activation-induced cytidine deaminase (AID) is involved in class switch recombination and deaminates cytosines to uracils in transcribed regions, which are then processed by DNA repair enzymes into a DSB. If DSBs coexist in the *IGH* and *C-MYC* genes, then they can recombine by interchromosomal translocation to produce an *IGH–C-MYC* fusion gene. Chr, chromosome.

then DNA replication is likely to be interspersed with the accumulation of structural variations throughout the development of a cancer, even though the structural variations may have accumulated over a fairly short period. This hypothesis is distinct from a phenomenon called chromothripsis, which comprises the formation of tens to hundreds of locally clustered structural variations that show a characteristic pattern of copy-number 'oscillations' (~2–3 copy-number states) with scattered losses of DNA fragments[85]. This type of structural variation is also locoregional but distinct from gene amplification, as it was arisen purportedly in a single cataclysmic moment in the history of a cancer. Both intrachromosomal and interchromosomal rearrangements arise from chromothripsis, which can lead to the formation of small circular marker chromosomes (double-minutes) that may subsequently amplify (that is, increase in copy number), particularly if they harbour an oncogene[85]. Recently, the term chromoplexy was given to the appearance of complex rearrangements that involve multiple chromosomes linked in a chain of rearrangements[98]. No specific pathophysiological mechanism has been implicated in this descriptive term.

Profound mechanistic insights can be gained from the detailed study of rearrangements that show marked colocalization with base substitution hypermutations — a phenomenon termed kataegis. Although all types of rearrangements have been described to harbour this unusual signature, which so far seems to be stochastic, it is the highly clustered base substitutions that show distinctive features: they comprise C·G→T·A transitions[2] and C·G→G·C transversions[9] with a marked predilection for a TpC or GpA sequence context and a striking strand coordination. Although the precise mechanism that underlies the kataegis signature is uncertain, an excess of these base substitutions at this specific sequence context has been found around induced DNA DSBs[19,99], which has prompted speculation that these clustered mutations occur at end-resected DSBs that expose ssDNA — the particular substrate of the APOBEC family of cytidine deaminases.

Chromosomal instability. Cancers are often characterized by chromosomal instability, which includes numerical or structural chromosomal aberrations. Historically, chromosomal instability is a feature that is defined at a macroscopic or chromosomal scale using techniques such as spectral karyotyping. Biologically, chromosomal instability has been attributed to replication stress in studies involving colorectal cancer cells[100] that arises from the activation of oncogenes such as *HRAS*, *CCNE1* (which encodes cyclin E), *MOS* and cell division cycle 6 (*CDC6*)[101–103]. These activated oncogenes induce the deregulation of cyclin-dependent kinase 2 (CDK2), which is involved in replication origin firing[104,105]. Interestingly, oncogene-induced replication stress has been shown to result in genetic instability and DSB formation specifically at fragile sites[106,107], which are hot spots for gene rearrangements[107]. Currently, it remains unclear how chromosomal instability translates to a genomic signature at the base-pair level.

Structural variation and immune loci. The generation of double-ended DSBs can be physiological. It is a necessary part of maturation at the *IG* locus of cells of the immune system. This deliberate activity may be achieved by V(D)J recombination-activating protein 1 (RAG1) and RAG2 (REF. 108), as well as by activation-induced cytidine deaminase (AID)-mediated class switch recombination[109] or somatic hypermutation[79]. Intriguingly, the role of these proteins can be appreciated as signatures in various haematological malignancies. For example, the RAG proteins, which show sequence specificity for a recombination signal sequence, underpin rearrangements between the *IGH* locus and the B-cell CLL/lymphoma 2 (*BCL2*) gene that drive follicular lymphoma[110] (FIG. 5c), whereas the AID protein is required for *C-MYC–IGH* chromosomal translocations that drive Burkitt's lymphoma[111,112] (FIG. 5d). In these malignancies, detailed analyses of the distribution of translocations in lymphocytes using genome-wide approaches have provided insights into the nonrandom nature of AID-mediated rearrangements[86–88]. These studies are further supported by observations in genome-sequenced haematological malignancies such as B-cell leukaemias and lymphomas[9]. Similar to kataegis, foci of substitutions are found to be coupled to rearrangements but, unlike kataegis, they are not stochastic; that is, they show recurrence at the *IGH* and *C-MYC* loci. They also show a preference for a purine preceding a mutated cytosine[9]; this sequence specificity differs from that of kataegis but is consistent with that of AID-mediated translocation. Breakpoint analyses have shown that MMEJ is involved in the ligation of the broken ends[86–88].

Conclusions

Each complex and multidimensional cancer genome may carry one or more mutational signatures (that is, the imprints of all of the mutational processes that have occurred throughout cancer development). The enduring mutational signatures in cancer genomes are the final physiological readout of the biology that has gone wrong throughout the development of the cancer, the readout of mutagenic damage from environmental or endogenous sources, as well as that of the repair and replicative processes that have been operative. The studies presented here show how technological advances in sequencing the human genome have led to a deeper appreciation of somatic mutational signatures in human cancers. By studying these enormous data sets in great detail, mechanistic insights can be gained. However, it must be highlighted that many recently discovered signatures are novel and remain to be understood. There is demand for experimental evidence even for signatures with clear candidate processes.

Several important observations should be highlighted. First, the overarching 96-element pattern of each signature is essentially identical between cancer samples of different patients, even of disparate cancer types, which suggests that similar processes are operating in different individuals. However, the individual somatic mutations that make up each signature in patient-specific cancers are highly variable between patients[9]. Second, some

Chromothripsis
An event with tens or hundreds of locally clustered rearrangements that result in distinct oscillations of copy-number states.

Chromoplexy
A rearrangement event that involves multiple chromosomes.

Kataegis
A base substitution hypermutation that comprises C·G→T·A transitions and C·G→G·C transversions with a predilection for a thymine preceding the mutated cytosine (that is, a TpC context); it usually macroscopically colocalizes with structural variation.

Chromosomal instability
A process that results in failure to maintain euploidy after mitosis and that is caused by either numerical or structural chromosomal aberrations.

Replication stress
A condition in which progression of a replication fork is hindered.

signatures seem to be the final readout of a deregulated pathway regardless of the precise somatic or germline mutation that underlies the perturbation (for example, the biallelic somatic and germline mutations of *BRCA1* and *BRCA2* in Signature 3). In these cases, knowledge of signatures could inform clinical decision making, for example, regarding potential sensitivity to therapeutics in the absence of precise genotypic information. The relationship between mutational signatures and clinical response to therapeutics requires investigation. Coupled to systematic characterization by experimental manipulation of model systems and detailed annotation of the resulting signatures, this will take us a step closer to more tailored treatments.

Given that mutational signatures are revealing the consequence of abrogated pathways, knowledge of the presence of a particular signature may enable targeting of the underlying mutational processes and thus provide a more successful path for cancer disease control. To this end, it is important for future work to determine the mutational processes that are still ongoing, either through serial biopsies from patients or through cell-line-based experiments. In addition, therapeutic strategies that selectively target processes responsible for specific signatures could complement current genotype-specific strategies. In the future, molecular genomic profiling should incorporate all mutations regardless of whether they are causative or consequential.

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Competing interests statement
The authors declare no competing interests.

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