Genomic instability — an evolving hallmark of cancer

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Abstract | Genomic instability is a characteristic of most cancers. In hereditary cancers, genomic instability results from mutations in DNA repair genes and drives cancer development, as predicted by the mutator hypothesis. In sporadic (non-hereditary) cancers the molecular basis of genomic instability remains unclear, but recent high-throughput sequencing studies suggest that mutations in DNA repair genes are infrequent before therapy, arguing against the mutator hypothesis for these cancers. Instead, the mutation patterns of the tumour suppressor *TP53* (which encodes p53), ataxia telangiectasia mutated (*ATM*) and cyclin-dependent kinase inhibitor 2A (*CDKN2A*; which encodes p16INK4A and p14ARF) support the oncogene-induced DNA replication stress model, which attributes genomic instability and *TP53* and *ATM* mutations to oncogene-induced DNA damage.

Hereditary non-polyposis colon cancer
An autosomal dominant disease that is characterized by increased susceptibility to colon carcinoma and other forms of cancer owing to an inherited defect in DNA mismatch repair genes.

DNA mismatch repair
The repair of DNA base-pair mismatches that arise as a result of replication errors or after exposure to several DNA damaging agents.

MYH-associated polyposis
An autosomal recessive disease that predisposes to colorectal cancer and is caused by germline mutations in the DNA repair gene *MYH*.

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Genomic instability is a characteristic of almost all human cancers, but at what stage of cancer development it arises and what its molecular basis is are questions to which we are only beginning to get answers. There are various forms of genomic instability. Most cancers have a form that is called chromosomal instability (CIN), which refers to the high rate by which chromosome structure and number changes over time in cancer cells compared with normal cells. Abnormal chromosome structures and numbers and abnormal mitoses associated with CIN were first visualized more than a hundred years ago[1,2]. Some of these chromosomal changes were seen in all cells of a tumour but others were not, suggesting that tumour cells are the progeny of a genetically unstable single cell, which continues to acquire chromosomal abnormalities over time[3,4]. The presence of CIN has also been confirmed in cancer cells grown in tissue culture[5].

Although CIN is the major form of genomic instability in human cancers, other forms of genomic instability have also been described. These include microsatellite instability (MSI; also known as MIN), a form of genomic instability that is characterized by the expansion or contraction of the number of oligonucleotide repeats present in microsatellite sequences[6,7], and forms of genomic instability that are characterized by increased frequencies of base-pair mutations[8].

Hereditary versus sporadic cancers

In hereditary cancers, the presence of both CIN and non-CIN forms of genomic instability has been linked to mutations in DNA repair genes. One of the best-documented examples is hereditary non-polyposis colon cancer (HNPCC; also known as Lynch syndrome), in which mutations in DNA mismatch repair genes lead to MSI[6,7]. Another example is hereditary MYH-associated polyposis, in which biallelic germline mutations in *MYH* (also known as *MUTYH*) — a DNA base excision repair (BER) gene — result in increased G•C to T•A transversion frequencies and cancer[8]. In hereditary cancers that are characterized by the presence of CIN, the genomic instability can also be attributed to mutations in DNA repair genes. For example, germline mutations in breast cancer susceptibility 1 (*BRCA1*), *BRCA2*, partner and localizer of BRCA2 (*PALB2*), BRCA1-interacting protein 1 (*BRIP1*), *RAD50*, Nijmegen breakage syndrome protein 1 (*NBS1*; also known as *NBN*), Werner syndrome helicase (*WRN*), Bloom syndrome helicase (*BLM*), RecQ protein-like 4 (*RECQL4*) and the Fanconi anaemia genes, all of which have been linked to the repair of DNA double-strand breaks (DSBs) or DNA interstrand cross links, predispose to the development of various cancers, including breast and ovarian cancer, leukaemias and lymphomas[9–11]. Finally, germline mutations in nucleotide excision DNA repair genes predispose to skin cancer[12].

The identification of mutations in DNA repair genes in hereditary cancers provides strong support for the mutator hypothesis, which states that genomic instability is present in precancerous lesions and drives tumour development by increasing the spontaneous mutation rate[4,13]. Proponents of the mutator hypothesis attribute the genomic instability in precancerous lesions to mutations in caretaker genes; that is, genes that primarily

function to maintain genomic stability[4,13,14]. Indeed, in inherited cancers, germline mutations targeting DNA repair genes are present in every cell of the patient's body. Thus, a single event — loss of the remaining wild-type allele — would lead to genomic instability and drive tumour development, as predicted by the mutator hypothesis.

The classical caretaker genes are DNA repair genes and mitotic checkpoint genes. The tumour suppressor gene *TP53*, which encodes p53, and the ataxia telangiectasia mutated (*ATM*) gene could also be considered as caretaker genes because they both function in the DNA damage response. However, as discussed below, *TP53* and *ATM* are subject to selective pressure for inactivation in cancer, whereas the classical caretaker genes are not. Because of this difference, when we refer to caretaker genes in this Review we do not include *TP53* and *ATM*.

Germline mutations in caretaker genes can explain the presence of genomic instability in inherited cancers. However, efforts to identify caretaker genes, the inactivation of which leads to genomic instability in sporadic (non-hereditary) cancers, have met with limited success[15]. For example, mutations in the mitotic checkpoint gene budding uninhibited by benzimidazole 1 (*BUB1*) can lead to CIN in experimental models, but in human cancers *BUB1* mutations are rare[16,17]. Furthermore, a systematic analysis of the sequences of 100 cell cycle checkpoint and DNA repair genes in early passage human colon cancer cell lines identified very few mutations[18].

Thus, unlike hereditary cancers, the molecular basis of genomic instability in sporadic cancers remains unclear. In this Review, we consider two models that could explain the presence of CIN — the main form of genomic instability — in sporadic cancers. The first is the mutator hypothesis described above and the second is the oncogene-induced DNA replication stress model for cancer development[19–23]. According to the latter model, CIN in sporadic cancers results from the oncogene-induced collapse of DNA replication forks, which in turn leads to DNA DSBs and genomic instability. These two models are reviewed in light of the recent high-throughput sequencing studies of human cancers, which have generated a wealth of information.

High-throughput sequencing studies

In the past few years, several consortia have begun sequencing the genomes of human cancers[24–29]. These efforts have identified thousands of mutations in primary cancers and early passage cancer cell lines or xenografts. Here, we first describe the sequencing studies that are relevant to this Review and then we discuss and analyse their findings in the context of understanding the mechanisms that lead to genomic instability in sporadic human cancers.

Analysing cancer samples for gene mutations. In one study, Vogelstein and his collaborators sequenced 18,191 genes in early passage xenografts or cancer cell lines obtained from 11 breast and 11 colorectal cancers[24,25]. In the breast cancers, mutations were found in 1,137 genes. When these genes were sequenced in an additional 24 breast cancers, mutations were found in 167 of them. In the colorectal cancers, 848 genes with mutations were identified in the initial discovery screen. In the validation screen, in which 24 additional colon cancers were sequenced, mutations were found in 183 of the 848 genes.

The same group of investigators also analysed the coding sequences of 20,661 genes in 24 advanced pancreatic adenocarcinomas[26] and in 22 glioblastomas[27]. The pancreatic adenocarcinomas were examined as early passage xenografts or cancer cell lines. Of the 22 glioblastomas, 7 were examined immediately after removal from the patient and 15 were first passaged as xenografts. Genomic deletions and amplifications in these cancer genomes were also identified using single nucleotide polymorphism arrays. In the pancreatic adenocarcinomas, 1,327 genes were mutated in at least one sample. In the glioblastomas, 685 genes were mutated at least once. In a subsequent validation phase, 39 genes were sequenced in an additional 90 pancreatic cancers and 21 genes were sequenced in an additional 83 glioblastomas, thus allowing a better estimate of the mutation frequencies of the genes identified in the initial screen to be frequently targeted in these cancers.

Glioblastomas were also studied by The Cancer Genome Atlas Research Network[28]. Primary tissue from 72 newly diagnosed, untreated glioblastomas and 19 treated cases was examined for mutations in 601 cancer-relevant genes. Genomic deletions and amplifications were also identified in these samples. In the treated and untreated cases combined (91 in total), 223 genes were mutated, 79 of which were mutated in more than one sample.

The largest study, in terms of the number of cancers examined, was carried out by many of the same groups comprising the Cancer Genome Atlas Research Network. In this study, 623 cancer-relevant genes were sequenced in 188 primary lung adenocarcinomas[29]. Mutations were identified in 356 genes, 193 of which were mutated more than once. Genomic deletions and amplifications were also identified.

A paucity of frequently mutated genes. In all of the high-throughput sequencing studies referred to above, few genes were found to be mutated, deleted and/or amplified at high frequencies[24–29]. Specifically, in each cancer type, about 4 genes were altered in more than 20% of the tumours analysed. The *TP53* tumour suppressor and DNA damage checkpoint gene was among the most frequently mutated genes in all tumour types examined. The remaining frequently deregulated genes encode either classical oncoproteins, such as the epidermal growth factor receptor (EGFR) and the small GTPase RAS, or tumour suppressor proteins, such as the cyclin-dependent kinase 4 inhibitor p16INK4A (encoded by cyclin-dependent kinase inhibitor 2A (*CDKN2A*)), the phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and neurofibromatosis type 1 (NF1), which antagonize the growth-promoting activities of oncoproteins. The identities of the frequently deregulated

Base excision repair
A pathway of DNA repair in which a single damaged base is excised by a DNA glycosylase.

Fanconi anaemia
A rare autosomal recessive disease that is characterized by developmental abnormalities, aplastic anaemia and increased susceptibility to solid and haematopoietic cancers.

Xenograft
A tissue or organ from one species transplanted into another species. The term is derived from the Greek 'Xeno', meaning foreign, and graft.

Glioblastoma
The most frequent and most malignant type of primary brain cancer. It is composed of poorly differentiated neoplastic astrocytes.

growth-regulating genes in these studies varied between tumour types[24–29]. For example, *RAS* mutations were frequent in pancreatic and lung adenocarcinomas but were essentially absent in glioblastomas, in which *PTEN*, *NF1* and *EGFR* mutations were the most prevalent.

Mutations targeting caretaker genes in sporadic cancer. The mutator hypothesis predicts that mutations affecting caretaker genes will be frequent and occur early in cancer development[4,13,14]. However, as mentioned above, targeted sequencing studies to look for mutations in known or predicted DNA repair and mitotic checkpoint genes failed to identify genes that were frequently mutated in sporadic human cancers[15–18], although it is possible that mutations in so far uncharacterized caretaker genes remain to be discovered.

Of the 5 high-throughput sequencing studies described above, 3 sequenced the coding sequences of 18,191–20,661 genes in carcinomas of the colon, breast and pancreas and in glioblastomas[24–27]. In these unbiased, genome-wide studies, mutations targeting caretaker genes were infrequent. Cumulatively in the 4 tumour types, representing 68 cancers in the discovery screen and 221 cancers in the following validation or prevalence screens, the most frequently mutated caretaker gene was Cockayne syndrome type B (*CSB*; also known as *ERCC6* and *RAD26*), a gene that encodes a chromatin remodelling factor that functions in transcription coupled-nucleotide excision repair (TC-NER). *CSB* was mutated in six cancers (TABLE 1). Four genes involved in the repair of DNA DSBs — *BRCA1*, *BRCA2*, meiotic recombination 11 (*MRE11*) and protein kinase, DNA-activated, catalytic polypeptide (*PRKDC*, which encodes the catalytic subunit of the Ser/Thr protein kinase DNA-PK, DNA-PKcs) — and one gene involved in the spindle assembly checkpoint (SAC), centromere-associated protein E (*CENPE*), were each mutated in two cancers. Finally, one non-synonymous mutation was found in the genes encoding FANCA, FANCD2, FANCG and FANCM (of the Fanconi anaemia DNA repair pathway); RAD51-like protein 3 (RAD51L3), RAD52, BRCA1-associated protein 1 (BAP1), PALB2 and WRN (which are involved in homologous recombination (HR) repair); X-ray repair cross-complementing 1 (XRCC1; which functions in BER); xeroderma pigmentosum group B-complementing protein (XPB; also known as ERCC3), XPF (also known as ERCC4), XPG (also known as ERCC5) and RAD23A (which are involved in NER); and TTK (also known as MPS1), ZW10 and kinetochore-associated protein 1 (KNTC1; which are components of the mitotic spindle assembly checkpoint) (TABLE 1).

Thus, the low frequency of mutations in known or suspected caretaker genes in these studies argues against the mutator hypothesis for sporadic cancers. Furthermore, the sequencing failed to identify novel putative caretaker genes that were frequently mutated in cancer. The only surprise was the presence of mutations targeting isocitrate dehydrogenase 1, which encodes a metabolic enzyme that catalyses the conversion of isocitrate to α-ketoglutarate, in 12% of the glioblastomas screened[24–27].

Similar results were obtained from analysis of a more limited set of about 600 genes, including many DNA repair and cell cycle checkpoint genes, in 188 lung adenocarcinomas and 91 glioblastomas[28,29]. In the lung adenocarcinomas, the non-homologous end joining (NHEJ) DNA repair gene *PRKDC* and the mismatch repair gene *MSH6* were mutated in six and four cases, respectively. The HR repair genes *BRCA2*, *BAP1* and BRCA1-associated RING domain 1 (*BARD1*) were mutated in two cases each. Last, the HR gene *BRCA1*, the BER gene *XRCC1*, the NER gene *XPD* (also known as *ERCC2*) and the mitotic checkpoint genes *BUB1* and *STK12* (also known as *AURKB*) were mutated in one case each (TABLE 1). In the glioblastomas, the frequency of mutations differed dramatically between the untreated and treated cases (TABLE 1). In the 72 untreated cases, only 2 mutations were found, one targeting *BRCA2* and the other targeting *MSH2*. In the 19 treated cases, 14 mutations were found, representing a 26-fold increase in mutation frequency compared with untreated cases. This difference suggests that mutations in caretaker genes in a specific tumour may be a late event, which might not contribute to the initial development of the tumour.

The results described above suggest that 3–31% of untreated sporadic human cancers have one or more mutations in a caretaker gene. More specifically, in the genome-wide studies, the frequencies ranged from 14–31%, depending on the tumour type, whereas in the two more focused studies the frequencies were 3% for untreated glioblastomas, 11% for untreated lung carcinomas and 37% for treated glioblastomas (TABLE 1). The higher frequencies seen in the genome-wide studies reflect, in part, the fact that more caretaker genes were examined. However, even taking this into account, the frequency of mutations is still higher, perhaps because the primary tumours in these studies were examined after being propagated as cell lines or xenografts[24–27]. Differences in the methods used to identify mutations could also account for the difference in mutation frequencies as the methods used to analyse genome-wide sequencing data are still under development[30,31].

Based on the above results, it could be concluded that genomic instability in many sporadic human cancers is not due to inactivation of caretaker genes (between 69–97% of cancers did not have mutations in caretaker genes in the various studies). Of course, such a conclusion is not without caveats. The mutation frequency may underestimate the frequency of gene inactivation because gene function can also be repressed by epigenetic mechanisms[32]. Alternatively, the mutation frequency may overestimate the frequency of gene inactivation. First, not all non-synonymous mutations compromise gene function. Some of these mutations may be passenger mutations; that is, mutations that do not drive cancer development[30,31]. Second, most, but not all[33], caretaker genes are recessive, which means that both alleles must be mutated for loss of function at the cellular level. When the mutations were acquired also needs to be considered. According to the mutator hypothesis, caretaker genes should be inactivated early in cancer development to establish conditions that allow mutations to accumulate

Transcription coupled-nucleotide excision repair
The branch of nucleotide excision repair — a pathway that repairs damaged bases by excision of a 25–30-nucleotide stretch of the DNA strand that contains the damaged base or bases — that repairs DNA lesions in the transcribed strands of active genes.

Spindle assembly checkpoint
The checkpoint that monitors the proper attachment of chromosomes to spindle microtubules.

Non-synonymous mutation
A mutation that results in an alteration of the amino acid sequence of a protein.

Fanconi anaemia DNA repair pathway
A pathway that primarily repairs DNA interstrand cross links.

Homologous recombination (HR) repair
The error-free repair of DNA DSBs, in which the broken DNA molecule is repaired using homologous sequences.

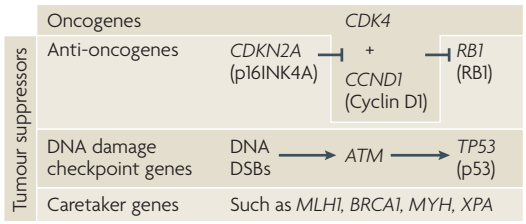
Non-homologous end joining
A pathway that repairs DNA DSBs by directly ligating the broken ends, without the need for a homologous template.

Table 1 | **Non-synonymous mutation frequencies in DNA repair and mitotic checkpoint genes in sporadic human cancers**

Gene*	Function	Cancer type						
		Breast‡	Colon‡	GB§	GB(Un)‖	GB(Tr)‖	Lung¶	Pancreas#
BRCA1	HR	2/35	0/11	0/22	0/72	0/19	1/188	0/24
BRCA2	HR	1/35	0/11	0/22	1/72	2/19	2/188	1/24
FANCA	HR	1/35	0/11	0/22	NE	NE	NE	0/24
FANCD2	HR	0/11	0/11	1/22	NE	NE	NE	0/24
FANCG	HR	0/11	1/35	0/22	NE	NE	NE	0/24
FANCM	HR	1/35	0/11	0/22	NE	NE	NE	0/24
FANCN (PALB2)	HR	0/11	0/11	0/22	NE	NE	NE	1/24
BAP1	HR	1/35	0/11	0/22	NE	NE	2/188	0/24
BARD1	HR	0/11	0/11	0/22	NE	NE	2/188	0/24
MRE11	HR	2/35	0/11	0/22	NE	NE	NE	0/24
RAD51L3	HR	0/11	0/11	1/22	NE	NE	NE	0/24
RAD52	HR	0/11	0/11	1/22	NE	NE	NE	0/24
WRN	HR	0/11	1/35	0/22	NE	NE	NE	0/24
PRKDC (DNA-PKcs)	NHEJ	0/11	0/11	2/22	0/72	2/19	6/188	0/24
MLH1	MMR	0/11	0/11	0/22	0/72	3/19	0/188	0/24
MSH2	MMR	0/11	0/11	0/22	1/72	1/19	0/188	0/24
MSH6	MMR	0/11	0/11	0/22	0/72	4/19	4/188	0/24
PMS1	MMR	0/11	0/11	1/22	0/72	0/19	NE	0/24
PMS2	MMR	0/11	0/11	0/22	0/72	1/19	NE	0/24
CSB (ERCC6 and *RAD26)*	NER	3/35	2/35	0/22	NE	NE	NE	1/24
XPB (ERCC3)	NER	1/35	0/11	0/22	NE	NE	NE	0/24
XPD (ERCC2)	NER	0/11	0/11	0/22	NE	NE	1/188	0/24
XPF (ERCC4)	NER	0/11	0/11	0/22	NE	NE	NE	1/24
XPG (ERCC5)	NER	0/11	0/11	1/22	NE	NE	NE	0/24
RAD23A	NER	0/11	0/11	1/22	NE	NE	NE	0/24
XRCC1	BER	0/11	1/35	0/22	NE	NE	1/188	0/24
BUB1	SAC	0/11	0/11	0/22	NE	NE	1/188	0/24
CENPE	SAC	2/35	0/11	0/22	NE	NE	NE	0/24
ZW10	SAC	0/11	0/11	1/22	NE	NE	NE	0/24
TTK (MPS1)	SAC	0/11	0/11	0/22	0/72	0/19	NE	1/24
KNTC1	SAC	0/11	0/11	1/22	NE	NE	NE	0/24
STK12 (AURKB)	SAC and cytokinesis	0/11	0/11	0/22	0/72	1/19	1/188	0/24
Number of mutations in caretaker genes		14/35	5/35	10/22	2/72	14/19	21/188	5/24
Number of cancers with mutations in caretaker genes		10/35	5/35	7/22	2/72	7/19	NA	5/24

BAP1, BRCA1-associated protein 1; *BARD1*, BRCA1-associated RING domain 1; BER, base excision repair; *BRCA*, breast cancer susceptibility gene; *BUB1*, budding uninhibited by benzimidazole 1; *CENPE*, centromere-associated protein E; *CSB*, Cockayne syndrome type B; *FANC*, Fanconi anaemia gene; GB(Tr), treated glioblastoma; GB(Un), untreated glioblastoma; HR, homologous recombination; *KNTC1*, kinetochore-associated protein 1; MMR, mismatch repair; *MRE11*, meiotic recombination 11; NA, information not available in REF. 29; NE, not examined; NER, nucleotide excision repair; NHEJ, non-homologous end joining; *PRKDC*, protein kinase, DNA-activated, catalytic polypeptide; *RAD51L3*, RAD51-like protein 3; SAC, spindle assembly checkpoint; *WRN*, Werner syndrome helicase; XP, xeroderma pigmentosum; XRCC1, X-ray repair cross-complementing 1. *For each gene (with alternative names provided in brackets), the number of mutations identified and the number of cancers examined is shown, and the total number of mutations in all caretaker genes examined is presented. Because some cancers had more than one mutation, the number of cancers with mutations in caretaker genes is lower than the number of mutations in caretaker genes. ‡Data from REF. 25. In the breast carcinoma study described in REF. 25, all three missense mutations in *CSB* were present in the same tumour. §Data derived from REF. 27. In the GB study described in REF. 27, one patient has been treated and his cancer had one missense mutation in *PRKDC*, one splice site mutation in *RAD23A* and one missense mutation in *ZW10*. ‖Data derived from REF. 28. In the GB study described in REF. 28, two of the three missense mutations in *MLH1* were present in the same treated tumour. ¶Data derived from REF. 29. #Data derived from REF. 26.

Box 1 | **Classes of tumour suppressor genes**



Tumour suppressor genes can be divided into distinct classes based on the primary function of the proteins they encode (see the figure). Anti-oncogenes, such as cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and retinoblastoma 1 (*RB1*), which encode p16INK4A and RB1, respectively, antagonize the growth-promoting activities of oncogenes, such as *CDK4* and *CCND1*, which encode CDK4 and cyclin D1, respectively. DNA damage checkpoint genes, such as ataxia telangiectasia mutated (*ATM*) and the tumour suppressor *TP53*, which encode ATM and p53, respectively, induce cell death or senescence in response to DNA damage or DNA replication stress. Caretaker genes (DNA repair genes and mitotic checkpoint genes, such as *MLH1*, breast cancer susceptibility 1 (*BRCA1*), *MYH* (also known as *MUTYH*) and xeroderma pigmentosum group A (*XPA*)) encode proteins that help to maintain genomic stability. Some tumour suppressors have more than one function and could fit in to more than one of the classes described here; thus, the distinction proposed here is based on primary function. DSB, double-strand break.

in growth-regulating genes. However, the higher frequency of mutations in caretaker genes in treated versus untreated glioblastomas[27,28] suggests that some mutations in caretaker genes may be a late event in cancer development and/or occur in response to therapy.

The premise, with the caveats listed above, that genomic instability in most sporadic human cancers is not caused by mutations in caretaker genes is consistent with mutations in caretaker genes usually being recessive. It has been argued that both alleles of a caretaker gene would have to be mutated before the genome becomes unstable; two such mutations before the establishment of genomic instability might be a very rare event[34]. The same argument can also explain why mutations in caretaker genes contribute to genomic instability in hereditary cancers. In these cases, one of the two alleles is already mutated in the germ line and, therefore, only one mutation is required for the genome to become unstable.

Oncogenes induce genomic instability

If mutations targeting caretaker genes are not responsible for genomic instability in many sporadic human cancers, as the high-throughput sequencing studies seem to indicate, then, because of its genetic basis[5], genomic instability is probably due to mutations in other genes. Genomic instability, specifically CIN, characterizes almost all sporadic human cancers[1–3]. Thus, an attractive hypothesis is that the most frequently mutated genes in human cancer are the ones responsible for the presence of genomic instability.

As mentioned above, the high-throughput studies suggest that very few genes are mutated, deleted and/or amplified at high frequencies in sporadic human cancers[24–29]. These are the *TP53* tumour suppressor and DNA damage checkpoint gene and genes that regulate cell growth either positively (such as the oncogenes *EGFR* and *RAS*) or negatively (such as the tumour suppressor genes *CDKN2A* and *PTEN*).

It has been argued that oncogenes do not induce genomic instability[35]. Furthermore, as *TP53* is a DNA damage checkpoint gene[36], its inactivation could be expected to result in genomic instability. Nevertheless, deletion of the *TP53* gene in mouse models and human cells does not lead to aneuploidy[37,38] and, in human precancerous lesions, genomic instability is present before the establishment of *TP53* mutations[20,21]. By contrast, activation of oncogenes, and more generally of growth signalling pathways, induces loss of heterozygosity and genomic instability in mammalian cells cultured *in vitro*, human xenografts, mouse models and even in yeast[20–23,39–46]. These findings have led to the formulation of the oncogene-induced DNA replication stress model[19]. According to this model, the mechanism by which activated oncogenes induce genomic instability involves DNA replication stress. Specific genomic sites, called common fragile sites, are particularly sensitive to DNA replication stress[47]. In human precancerous lesions and several experimental systems in which oncogenes have been activated, genomic instability preferentially affects common fragile sites[20–23,48]. The high frequency of *TP53* mutations in human cancers could also be in response to oncogene-induced DNA damage. In precancerous lesions that typically retain wild-type p53 function, the oncogene-induced DNA damage elicits p53-dependent apoptosis and/or senescence, which limits growth of the lesion. When the function of p53 is lost, cells can escape its apoptotic and/or senescence effects, and the precancerous lesion can become cancerous[20–23].

It is important to note that the oncogene-induced DNA replication stress model distinguishes *TP53* from most other tumour suppressor genes. Whereas the protein product of *TP53*, p53, is a DNA damage checkpoint protein that responds to oncogene-induced DNA damage, most other tumour suppressors function in the same growth signalling pathways as oncogenes. For example, the tumour suppressor gene *PTEN* encodes a phosphatase that antagonizes the kinase encoded by the oncogene phosphoinositide 3-kinase-α (*PI3KA*) (REF. 49). Similarly, the p16INK4A protein product of the *CDKN2A* tumour suppressor gene directly inhibits the kinases encoded by the oncogenes *CDK4* and *CDK6* (REF. 50). The caretaker genes[4,13,14], which are mostly DNA repair genes, can be considered as a third class of tumour suppressor gene as their primary function is neither to affect cell growth nor to induce apoptosis or senescence in response to DNA damage (BOX 1).

Common fragile site
A chromosomal region that is prone to breakage after inhibition of DNA replication.

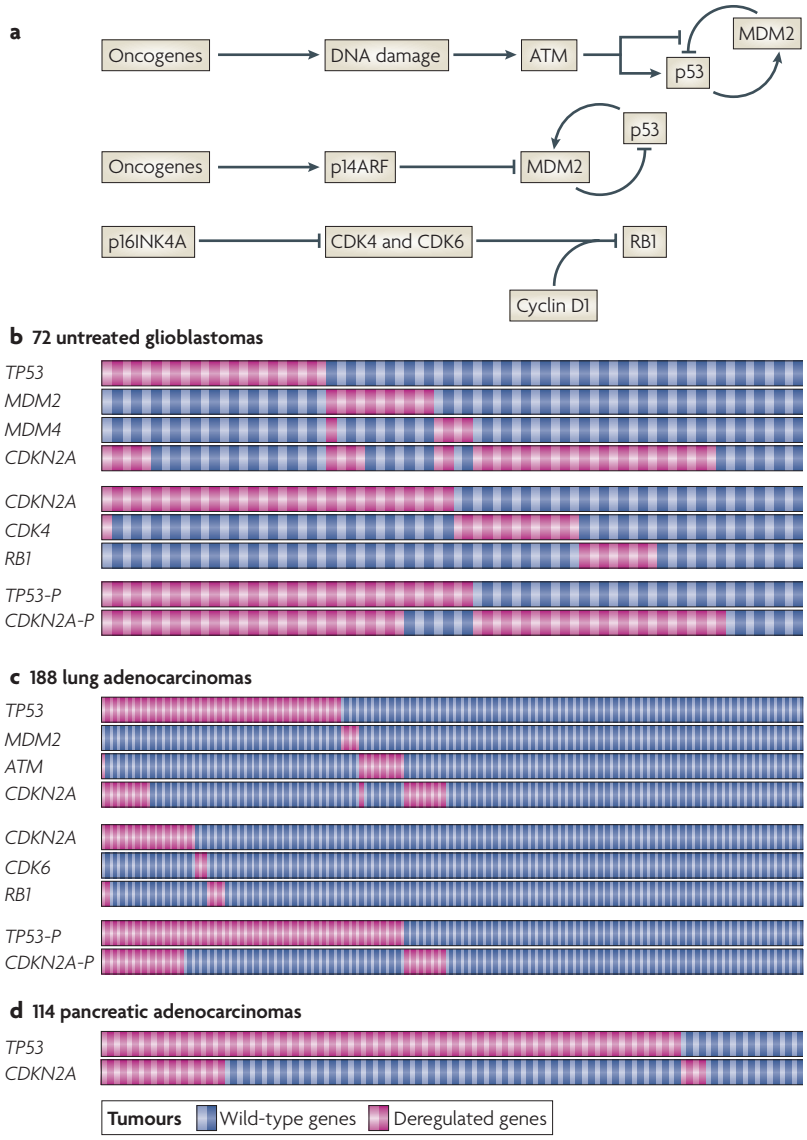


Figure 1 | **Mutations in p53 pathway genes are mutually exclusive with *ATM*, but not *CDKN2A*, mutations. a** | Key signalling pathways involving p53 (which is encoded by the tumour suppressor *TP53*) and p16INK4A and p14ARF (which are encoded by cyclin-dependent kinase inhibitor 2A (*CDKN2A*)). According to the oncogene-induced DNA replication stress model, oncogenes induce DNA replication stress and DNA double-strand breaks (DSBs), which activate ataxia telangiectasia mutated (ATM). ATM activates p53 and also disrupts the interaction of p53 with the E3 ubiquitin ligase MDM2, thereby inhibiting the MDM2-dependent degradation of p53. Another model proposes that oncogenes activate p14ARF, which inhibits MDM2, thereby also leading to p53 stabilization and activation. p16INK4A is an inhibitor of cyclin dependent kinase 4 (CDK4) and CDK6, which, in complex with cyclin D1, inhibit the retinoblastoma 1 (RB1) tumour suppressor protein. **b** | Correlation of the deregulation of *TP53* (by mutation or deletion), *MDM2* (by gene amplification or mutation), *MDM4* (by gene amplification or mutation), *CDKN2A* (by homozygous deletion), *CDK4* (by gene amplification) and *RB1* (by mutation) to each other, and of the *TP53* pathway (*TP53*-P) genes (*TP53*, *MDM2* and *MDM4*) to the *CDKN2A* pathway (*CDKN2A*-P) genes (*CDKN2A*, *CDK4* and *RB1*), in 72 untreated glioblastomas. The data were derived from REF. 28. **c** | Correlation of the deregulation of the *TP53* (by mutation), *MDM2* (by gene amplification), *ATM* (by mutation), *CDKN2A* (by deletion or mutation), *CDK6* (by gene amplification) and *RB1* (by mutation) genes to each other, and of the deregulation of *TP53*-P genes (*TP53*, *MDM2* and *ATM*) to *CDKN2A*-P genes (*CDKN2A*, *CDK6* and *RB1*) in 188 lung adenocarcinomas. The data were derived from REF. 29. **d** | Correlation of the mutation of *TP53* to the mutation of *CDKN2A* in 114 advanced pancreatic adenocarcinomas. The data were derived from REF. 26.

TP53 inactivation pathways

The high-throughput sequencing studies of human cancers cannot provide direct evidence that genomic instability is due to oncogene-induced DNA damage. However, these studies can be used to test a key point of the oncogene-induced DNA damage model; that is, that the selection for *TP53* mutations in cancer is due to oncogene-induced DNA damage (FIG. 1a). An alternative pathway by which *TP53* mutations can be selected in the absence of DNA damage involves the oncogene-induced expression of p14ARF, which is encoded by *CDKN2A* and inhibits MDM2 — the E3 ubiquitin ligase that targets p53 for ubiquitin-mediated degradation[51] (FIG. 1a).

If *TP53* mutations are selected in cancer owing to oncogene-induced DNA damage, then mutations in DNA damage response genes that function in the same pathway as *TP53* should be present in cancer. Furthermore, mutations in *TP53* and these other DNA damage response genes should be mutually exclusive, because either mutation would inactivate the pathway. Reciprocally, if *TP53* mutations are selected by high levels of p14ARF, then mutations targeting the genes encoding p14ARF and p53 would be mutually exclusive.

We first examined whether the data from the high-throughput sequencing studies were of sufficient quality to show mutually exclusive mutations of genes functioning in the same pathway. In the glioblastoma and lung adenocarcinoma studies, for which both mutation and gene copy number data were available from several cancers[28,29], *TP53* mutations and the amplification of *MDM2* and *MDM4* were mutually exclusive (FIGS 1b,c), consistent with the well-documented roles of MDM2 and MDM4 (an MDM2 homologue) in targeting p53 for ubiquitin-dependent degradation[52,53].

We then analysed the presence of *TP53* mutations in relation to inactivation of *CDKN2A*. *CDKN2A* encodes both p16INK4A and p14ARF through alternative reading frames (ARFs)[54]. In many cancers, the entire *CDKN2A* gene is deleted, resulting in loss of both p14ARF and p16INK4A expression. The high-throughput sequencing studies did not show mutually exclusive inactivation of *CDKN2A* and *TP53* in any of the cancer types examined (FIGS 1b,c,d). As *CDKN2A* encodes two proteins, the interpretation of these data is not straightforward. Interestingly, however, deregulation of *CDKN2A* was mutually exclusive with deregulation of *CDK4*, *CDK6* and retinoblastoma 1 (*RB1*) (FIGS 1b,c), suggesting that the inactivation of p16INK4A may be the driving force for the *CDKN2A* deletions, as p16INK4A functions in the same pathway as CDK4, CDK6 and RB1 (REFS 50,55) (FIG. 1a).

Of the DNA damage response genes that function upstream of *TP53*, *ATM* (which encodes a kinase that phosphorylates p53 in response to DNA damage) seems to be the gene that is most frequently mutated in human cancers[29,56]. However, *ATM* mutations are found only in specific cancer types. In the high-throughput sequencing studies discussed above, *ATM* mutations were identified only in lung adenocarcinomas. Interestingly, the *ATM* mutations were mutually exclusive with *TP53* mutations and *MDM2* amplification (FIG. 1c), suggesting

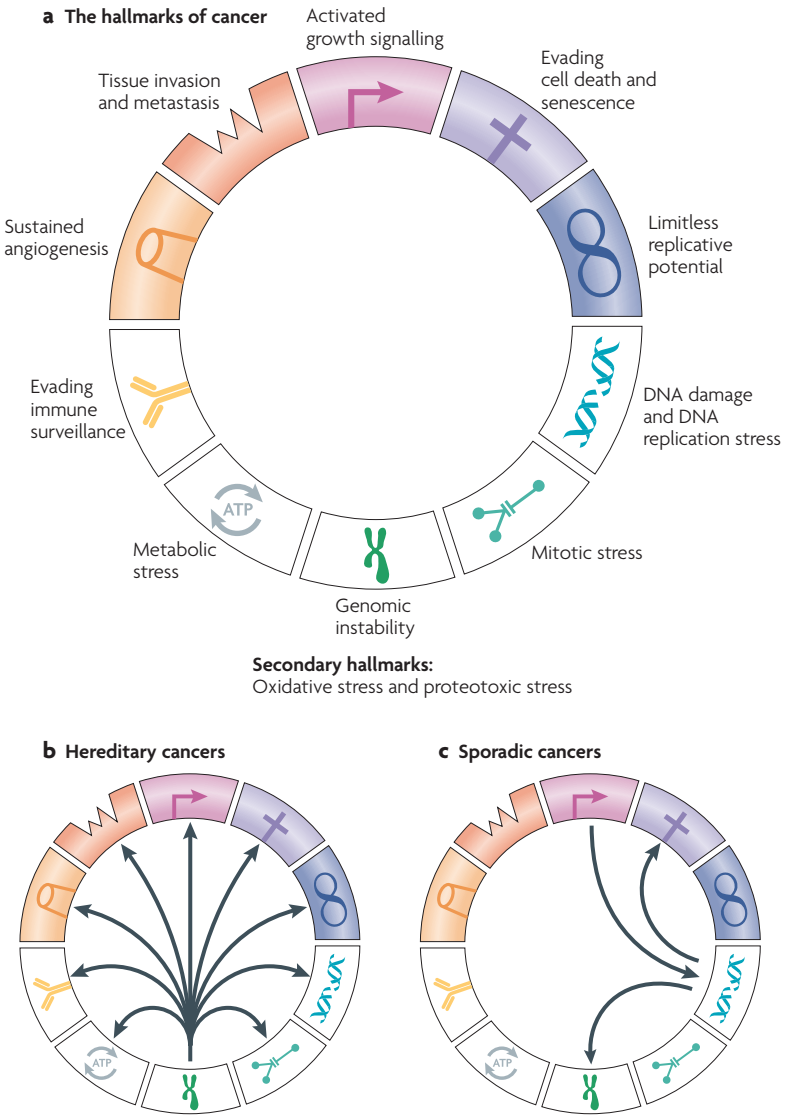


Figure 2 | **Genomic instability as a hallmark of cancer. a** | A proposed revision of the hallmarks of cancer to include genomic instability, and to consolidate the self-sufficiency in growth signals and insensitivity to anti-growth signals into the single hallmark of activated growth signalling. The secondary hallmarks (oxidative stress and proteotoxic stress) are shown separately. **b** | The temporal order by which the hallmarks are acquired in hereditary cancers. The establishment of genomic instability is probably the initiating event, which then facilitates the establishment of all the other hallmarks. **c** | The temporal order by which the hallmarks are acquired in sporadic (non-hereditary) cancers. Deregulation of growth-regulating genes can be the initiating event. This leads to DNA damage and DNA replication stress, which, in turn, lead to genomic instability and selective pressure for tumour suppressor p53 (*TP53*) inactivation. Loss of p53 function allows evasion from cell death, whereas the genomic instability provides a fertile ground for additional mutations that lead to the establishment of the remaining hallmarks, as in hereditary cancers. Figure in part **a** is modified, with permission, from REF 59 © (2009) Elsevier.

Proteotoxic stress
A cellular stress that is due to the accumulation of misfolded proteins arising from disequilibrium in the protein homeostatic machinery.

that in lung cancer these three genes constitute one functional pathway and that DNA damage is the signal that selects for p53 inactivation. Thus, the analysis of the high-throughput sequencing studies described above supports the oncogene-induced DNA replication stress model.

Genomic instability — a cancer hallmark

Ten years ago, Hanahan and Weinberg described six functional capabilities of cancers that they called hallmarks of cancer[57]. These six hallmarks were: self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, sustained angiogenesis, tissue invasion and metastasis, and unlimited replicative potential. Examples of the molecular basis of these hallmarks were also provided. For instance, mutations in genes that regulate growth can underlie the self-sufficiency in growth signals[57]. The concept was also introduced that mutations leading to the hallmarks did not have to be acquired in any specific order. Finally, genomic instability was considered separately from the six hallmarks, in that it is not a functional capability of cancer *per se* but a property that enables the acquisition of the hallmarks[57].

Recently, additional hallmarks of cancer have been proposed. These include evading immune surveillance[58] and five additional hallmarks relating to the presence of stress in cancer, namely: DNA damage and DNA replication stress, oxidative stress, mitotic stress, proteotoxic stress and metabolic stress[59]. These five new hallmarks are qualitatively different from the original hallmarks in that they do not describe functional capabilities of cancers but rather the state of cancer cells, which is characterized by the presence of various stresses[19,59].

The expansion of the concept of hallmarks to include states of cancer cells warrants, in our opinion, the inclusion of genomic instability as one of the hallmarks (FIG. 2a). Genomic instability is present in all stages of cancer, from precancerous lesions, even before *TP53* mutations are acquired[20,21], to advanced cancers[3–5].

The high-throughput sequencing studies reviewed in this article may suggest even further modifications of the cancer hallmarks. It seems that in all examined sporadic cancers there are very few genes that are mutated at high frequency[24–29], and these are genes that encode proteins that function in growth signalling (oncogenes and anti-oncogenes) or in the DNA damage checkpoint (such as *TP53*). The observation that only two classes of genes are frequently mutated in cancer is in contrast to the numerous described hallmarks. We therefore propose that some hallmarks can be consolidated. Specifically, mutations targeting the growth-regulating genes can explain two hallmarks of cancer: the self-sufficiency in growth signals and the insensitivity to anti-growth signals. Inactivation of *RB1* was proposed as an example of a mutation that facilitates insensitivity to anti-growth signals[57]. In contrast, amplification of the oncogenes *CDK4* or *CCND1* (the gene encoding cyclin D1, which is required for the kinase activity of CDK4) are considered as genetic changes that facilitate self-sufficiency in growth signals. However, *CDK4* and *CCND1* function in the same pathway as *RB1* (BOX 1; FIG. 1a). Thus, it is hard to envision that genetic changes that affect the same pathway in the same way, such as *RB1* inactivation and *CDK4* or *CCND1* amplification, give rise to different hallmarks. We therefore propose that the two hallmarks, self-sufficiency in growth signals and insensitivity to anti-growth signals, be consolidated into one, called activated growth signalling (FIG. 2a).

Another way to tackle the numerous hallmarks is to consider that some seem to be secondary to others. For example, proteotoxic stress may be secondary to aneuploidy, which in turn is a manifestation of genomic instability, and oxidative stress may be secondary to oncogenic signalling and metabolic stress[59]. Based on these considerations, we propose that the secondary hallmarks be presented separately from the primary hallmarks (FIG. 2a).

The high frequency of mutations targeting *TP53* also cannot be ignored when considering the hallmarks of cancer. The previously described hallmark of evading apoptosis fits well with the oncogene-induced DNA replication stress model, which posits that oncogene-induced DNA damage activates *TP53* and leads to apoptosis[19]. However, since oncogene-induced DNA damage also induces senescence[22,23], this hallmark could be expanded to include senescence, and possibly even other forms of p53-mediated cell death, such as autophagy[60,61] (FIG. 2a).

Finally, the question remains whether the acquisition of hallmarks occurs in any specific order, or in no specific order as originally proposed[57]. As described above, hereditary cancers are often characterized by the presence of mutations in DNA repair genes, such as *BRCA1*, *BRCA2*, *MSH2* and *MYH*, which leads to genomic instability[6–12]. Thus, in accordance with the mutator hypothesis[13], the presence of genomic instability in hereditary cancers probably precedes the acquisition of mutations in oncogenes and tumour suppressor genes and therefore precedes the acquisition of the other hallmarks (FIG. 2b). By contrast, in sporadic cancers, the high-throughput sequencing studies suggest that caretaker genes might not be frequently inactivated early in cancer development (TABLE 1). Instead, the first hallmark to be acquired in sporadic cancers might be activated growth signalling, owing to mutations in oncogenes or anti-oncogenes (FIG. 2c). The DNA replication stress that is associated with oncogene activation could then lead to genomic instability and the selection for *TP53* mutations, which results in cells evading cell death and senescence. Such an ordered acquisition of hallmarks would be consistent with the temporal order in which mutations in oncogenes, anti-oncogenes and *TP53* are acquired in colon cancer[62].

Conclusions and perspectives

It is evident from this Review that our understanding of genomic instability in cancer is still very limited. Its molecular basis is well understood in hereditary cancers, in which it has been linked to mutations in DNA repair genes. By contrast, the molecular basis of genomic instability in sporadic cancers is much less well defined. The high-throughput sequencing studies suggest that mutations in DNA repair or other caretaker genes probably do not account for the presence of genomic instability in many sporadic cancers. An alternative is that oncogene-induced DNA replication stress is responsible for the presence of genomic instability in these tumours. However, other alternatives are also possible, such as instability owing to telomere erosion[63]. Thus, further work is needed to understand the molecular basis of genomic instability in cancer.

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Competing interests statement
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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
ATM | *BARD1* | *BLM* | *BRCA1* | *BRCA2* | *BRIP1* | *BUB1* | *CCND1* | *CDK4* | *CDK6* | *CDKN2A* | *CENPE* | *CSB* | *MDM2* | *MDM4* | *MSH2* | *MSH6* | *MRE11* | *MYH* | *NBS1* | *PALB2* | *PRKDC* | *RAD50* | *RB1* | *RECQL4* | *STK12* | *TP53* | *WRN* | *XPD*
UniProtKB: http://www.uniprot.org
BAP1 | EGFR | FANCA | FANCD2 | FANCG | FANCM | KNTC1 | NF1 | p14ARF | p16INK4A | PTEN | RAD23A | RAD51L3 | RAD52 | TTK | XPB | XPE | XPG | XRCC1 | ZW10

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