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REVIEW ARTICLE

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Genomic destabilization and its associated mutagenesis increase with senescence-associated phenotype expression

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Abstract

Cancer develops through multiple rounds of clonal evolution of cells with abrogated defense systems. Such clonal evolution is triggered by genomic destabilization with associated mutagenesis. However, what increases the risk of genomic destabilization remains unclear. Genomic instability is usually the result of erroneous repair of DNA double-strand breaks (DSB); paradoxically, however, most cancers develop with genomic instability but lack mutations in DNA repair systems. In this manuscript, we review current knowledge regarding a cellular state that increases the risk of genomic destabilization, in which cells exhibit phenotypes often observed during senescence. In addition, we explore the pathways that lead to genomic destabilization and its associated mutagenesis, which ultimately result in cancer.

KEYWORDS

cell cycle, DNA replication, genetic instability, genomic instability

1 | INTRODUCTION

Most cancers develop genomic instability, which can be categorized as either chromosomal instability (CIN) or microsatellite instability (MSI).[1] CIN encompasses a wide variety of chromosomal abnormalities, including chromosome-number alterations (ie, aneuploidy) and chromosomal rearrangements,[2,3] whereas MSI is defined as changes in the lengths of microsatellite fragments containing short repetitive sequences.[4] Mismatch repair (MMR) status is a major determinant of whether CIN or MSI is induced; MSI is more likely to develop in cells with MMR deficiency.[5] Both CIN and MSI are induced through erroneous repair of DNA double-strand breaks (DSB) arising due to replication stress.[6]

Cancer develops as a consequence of mutations in cancer-driver genes. However, recent studies have illustrated that mutations, including those in cancer-driver genes, accumulate in association with age, even in pathologically normal organs.[7,8] This indicates that cancer development is not directly triggered by those mutations, raising the question of what the actual trigger might be. Recent studies have suggested that one trigger is genomic destabilization. Indeed, cancer development is tightly associated with accumulation of genomic abnormalities, which are rarely observed in normal organs even at advanced ages but are widely observed after tumorigenic progression.[9,10] In addition, a recent in vitro study revealed that genomic destabilization is associated with mutagenesis and acts as a trigger for clonal evolution of cells with defects in defense systems such as the ARF/p53 pathway.[6]

In this manuscript, we review the current knowledge about the cellular backgrounds and mechanisms that increase the risk of genomic destabilization (ie, the genomic instability induction associated with CIN and MSI) and other pathways that induce genomic destabilization-associated mutagenesis.

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2 | RISK OF GENOMIC DESTABILIZATION AND THE ASSOCIATED CELLULAR STATE

2.1 | Genomic instability and cancer risk

Genomic instability caused by erroneous repair of DSB is associated with cancer development. This phenomenon is clearly illustrated in cells harboring mutations in DNA repair systems. For example, in cells with mutations in the homologous recombination (HR) factors BRCA1 or BRCA2, genomic destabilization is strongly induced, predisposing the cells toward carcinogenesis.[11,12] Paradoxically, however, most cancers do not harbor any background mutations in DNA repair systems, despite the association between cancer development and genomic instability.[13,14] This poses the question of what increases the risk of genomic destabilization in normal cells.

2.2 | DNA double-strand breaks accumulate when normal cells exhibit senescence-associated phenotypes

Recent reports have revealed that the DSB repair-defective backgrounds that jeopardize genomic stability are more likely to appear when cells show senescence-associated phenotypes. Cellular senescence, defined as irreversible cell-cycle arrest, is generally induced in association with a series of phenotypes, including persistent DSB accumulation, increased expression of p16Ink4A and the senescence-associated secretory phenotype, and increased activity of senescence-associated β-Gal.[15,16] In fact, normal cells can accumulate persistent DSB that induce senescence in vitro and aging in vivo.[15] Although repair factors localize to these DSB, they tend to persist there without completing repair, suggesting repair deficiency.[17,18] Indeed, those cells are specifically defective in the repair of replication stress-associated DSB, which can cause genomic destabilization.[19] In addition, cells that accumulate DSB are likely predisposed to genomic destabilization.[20,21]

Normal cells generally undergo growth arrest after serial proliferation (Figure 1A). The growth-arrested cells can be divided into those that are in a quiescent state (Figure 1B) and those that are in a state characterized by senescence-related phenotypes and DSB. Cells in the former state arrest cell-cycle progression without DSB, as has been widely observed in the cells of healthy organs preserving homeostasis, such as the liver. By contrast, cells in the latter state are often observed in aging organs. These latter cells might not all be in a uniform state, because DSB accumulate as senescence progresses. Therefore, some cells could be in a pre-senescent state (Figure 1C) and others in a canonical senescent state (Figure 1D). Genomic destabilization likely occurs when cells accumulate DSB and are still progressing the cell cycle.[6] Escaping from senescence, clonal evolution of cells with abrogated defense systems will be induced among a genomic destabilized cell (Figure 1: see cells developing to the state shown in Figure 1E).

The risk of cancer generally increases with age and especially with genomic instability.[22,23] The main cause of cancer is probably genomic destabilization. Even in normal cells with functional DNA repair systems, the risk of genomic destabilization increases with senescence-associated phenotype expression. First, senescence-associated phenotypes are generally induced in response to DSB when the risk of genomic destabilization is elevated.[9,10] Second, these phenotypes are

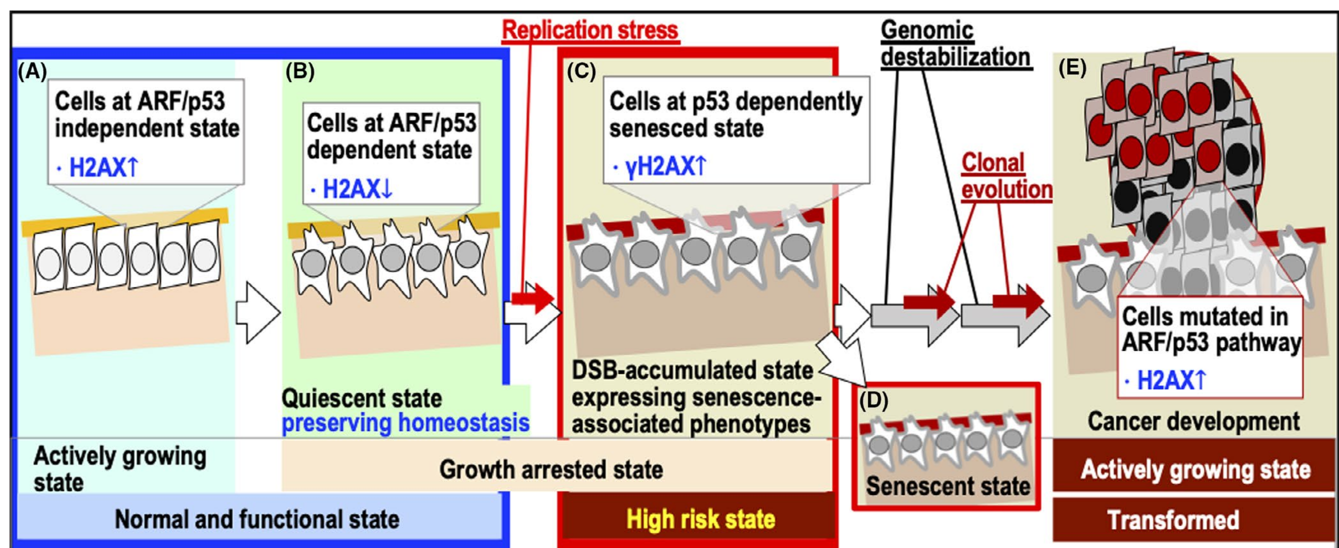

FIGURE 1 Alterations of cellular state in normal cells. Normal cells (A) eventually undergo growth arrest (B-D) after serial proliferation. Growth-arrested cells can continuously preserve the quiescent state (B), as often observed in normal organs preserving tissue homeostasis. However, cells in this state are sensitive to exogenous growth stimuli that enforce cell-cycle progression, which leads to the accumulation of replication stress-associated DNA double-strand breaks (DSB) (C) in cells that exhibit senescence-associated phenotypes. In situations where cells escape from the induction of the senescent state (D), genomic destabilization could be triggered by those DSB, further increasing the risk of clonal evolution of cells with abrogated defense systems. Cancer develops after multiple rounds of clonal evolution (E)

further induced in response to increased amounts of cytosolic DNA, a consequence of chromosomal mis-segregation caused by genomic destabilization.[24] Given that genomic destabilization can trigger clonal evolution of cells with abrogated defense systems,[6] cells with accumulated DSB and senescence-associated phenotypes could be major contributors to cancer development, which usually occurs in the elderly.

2.3 | Repair-defective cellular background

It remains unclear how senescent cells could become defective in repairing DSB. This phenomenon is at least partly due to a reduction in the H2AX level: H2AX, which mediates the damage response by forming γH2AX (phosphorylated H2AX at S139) foci at sites of damage,[25] is significantly downregulated when the growth rate of normal cells slows.[26] This reduction in H2AX level mediates two separate cellular phenotypes, formation of the quiescent cellular state and induction of a repair-defective state.[27] Cells that express very little H2AX can remain continuously quiescent (Figure 1B), like cells in normal organs under tissue homeostasis.[26] However, such cells are sensitive to exogenous growth stimuli that enforce cell-cycle progression, which results in the accumulation of replication stress-associated DSB and an increased risk of genomic destabilization (Figure 1C).[6,9] Thus, the H2AX-diminished state is associated with preservation of homeostasis but is simultaneously associated with a repair-defective background, posing the risk of future genomic destabilization (Figure 1B). This is supported by the phenotypes of H2AX-knockout (KO) cells, which exhibit elevated genomic instability; however, H2AX-KO mice are not predisposed to cancer.[28]

Although changes in the levels of H2AX and γH2AX are closely associated with the risk of genomic destabilization, these changes do not explain all of the genomic instability phenotypes observed in cancer cells. For example, cancer cells can recover H2AX expression, but often continue to exhibit higher rates of genomic changes,[26] implying the involvement of another mechanism that further increases the risk of genomic destabilization.

2.4 | Role of ARF/p53 in the formation of the quiescent state

As illustrated in an in vitro model, formation of the H2AX-diminished quiescent state is regulated by both ARF and p53 (Figure 1B). Therefore, this state can only be induced in normal cells, and not in cancer cells or cells transformed in vitro (Figure 1E).[26,29] Given that the H2AX-diminished state is widely observed in normal organs at steady state, quiescence is likely to be associated with preservation of homeostasis and cancer suppression.[26]

Among the targets of p53, those involved in cancer suppression remain unclear. Given that most cancers without mutations in *TP53* develop following loss of the *CDKN2A* gene, which encodes ARF,[30,31] the cancer-suppressive function of p53 is probably ARF-dependent. However, most p53 targets currently known were discovered in cancer cells harboring mutations in ARF, implying that the role of p53 in cancer suppression might be distinct from the identified p53 targets. In fact, most currently known p53 targets are likely to be involved in acute damage responses but are dispensable for the primary cancer-suppression effect.[32-34] Importantly, the ARF-dependent cancer-suppression role by p53 may involve the H2AX-diminished quiescent state induction, because this state can only arise when both ARF and p53 are correctly regulated (Figure 1B). However, it remains unclear how the ARF/p53 pathway mediates H2AX downregulation.

3 | GENOMIC DESTABILIZATION TRIGGERED BY REPLICATION STRESS

3.1 | Replication stress-triggered chromosomal instability induction

Mismatch repair (MMR)-proficient cells usually become defective in the repair of replication stress-associated DSB and develop senescent-associated phenotypes when the risk of CIN is elevated.[15] Such CIN-type genomic instability is induced through erroneous repair of those DSB,[2,19] which are primarily targeted by factors involved in HR. A wide variety of genomic alterations arise in the resultant cells, including ploidy abnormalities,[5] chromosome rearrangements and losses,[35,36] gene amplifications and deletions,[37] loss of heterozygosity (LOH),[38-40] and cytosolic DNA (ie, micronuclei).[41,42]

One outstanding question is how these multiple types of genome-wide alterations are induced. The answer probably has to do with the repair-pathway switch (Figure 2). In fact, although replication stress-associated DSB are the primary target of HR, most genomic rearrangements are joined through the error-prone non–homologous end joining (NHEJ) pathway,[43,44] suggesting a switch from HR to NHEJ (Figure 2A). This could be the result of cell-cycle carryover of DSB, as seen during tetraploidization.[19] Replication stress-associated DSB arise in cells displaying senescence-associated phenotypes, and are, therefore, often carried over into the M phase.[19] Such carryover can result in tetraploidization because mitotic DSB cause chromosomal mis-segregation, leading to failure of cytokinesis.[19,45] Importantly, this process could also cause the HR–NHEJ switch, because HR does not operate during G1 phase when DSB are usually repaired by NHEJ.[46,47] After the collapse of HR intermediates at entry into G1 phase, the DSB end must remain free until a partner DSB end is paired by factors involved in NHEJ. This provides the necessary time window for the generation of genome-wide structural variants such as chromosomal translocations and deletions. Chromosomal translocations associated with DSB carryover have been demonstrated in vitro.[48]

DNA double-strand breaks carryover into M and G1 phases mediates other CIN-associated genomic alterations as well.

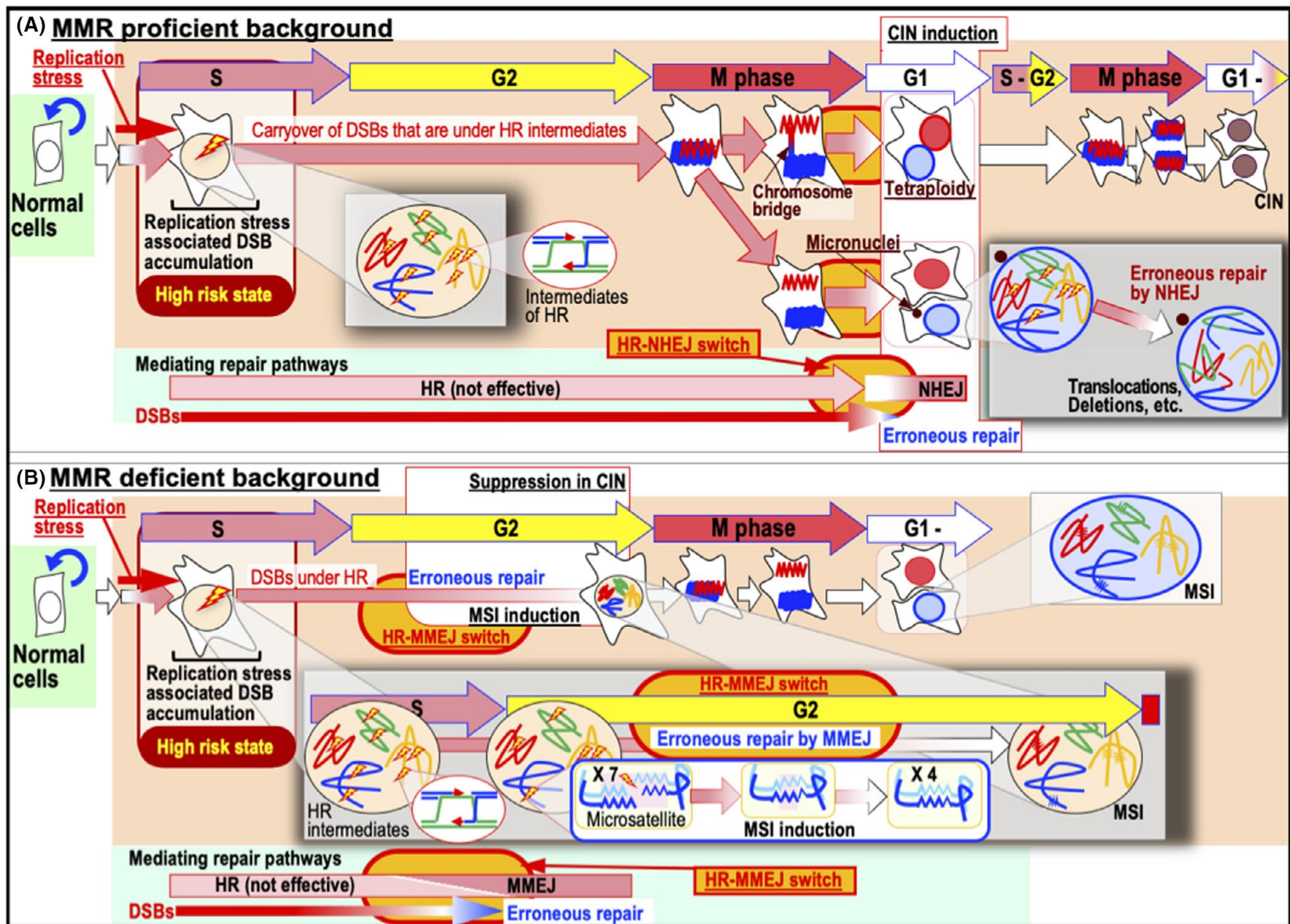

FIGURE 2 Replication stress triggers induction of chromosomal instability (CIN) or microsatellite instability (MSI). Cells become sensitive to replication stress when they exhibit senescence-associated phenotypes. Both CIN and MSI are triggered by replication stress-associated DNA double-strand breaks (DSB) that are primarily targeted by repair factors involved in HR, which is not active in the senescent state. (A) In a mismatch repair (MMR)-proficient background, those DSB are persistent but eventually undergo erroneous repair by non–homologous end joining (NHEJ), which can induce multiple types of chromosomal abnormalities, such as aneuploidy (tetraploidy), chromosomal translocations and deletions, and formation of micronuclei. (B) Under an MMR-deficient background, the DSB are erroneously repaired by MMEJ. During this process, MSI is induced and CIN is suppressed

Micronuclei are induced in association with mitotic DSB-mediated chromosomal mis-segregation, resulting in split chromosomal fragments.[49] Besides tetraploidization, aneuploidy is also widely induced during this process through imbalanced chromosomal segregation and loss of DNA due to micronuclei formation.[49,50] In addition, because HR does not operate effectively during CIN induction, LOH could also be induced through aberrant HR between allelic chromosomal loci.

3.2 | Replication stress-triggered microsatellite instability induction

microsatellite instability (ie, insertion/deletion errors specifically induced at repetitive loci) is widely observed in MMR-deficient cancer cells.[1] It was once thought that MSI was induced by replication errors under MMR deficiency.[51-53] However, recent studies suggest otherwise. In fact, MSI is triggered by replication stress-associated DSB, as an alternative to CIN (Figure 2B).[6,54] Replication stress-associated DSB in MMR-proficient cells persist continuously throughout S and M phases (Figure 2A), whereas DSB in MMR-deficient cells are primarily recognized by HR factors, but are effectively repaired by microhomology-mediated end joining (MMEJ) concomitant with induction of MSI[6] (Figure 2B). Microsatellite loci (ie, short repetitive sequence patches) are naturally advantageous for microhomology annealing and associated end joining; however, multiple annealing positions could be used, including those with small insertions or deletions, resulting in MSI induction.[55-57] Because this results in DSB elimination, MSI induction is associated with suppression of CIN.[6]

Although it remains unclear how the HR–MMEJ switch is specifically induced in an MMR-deficient background, it is probably associated with complex formation.[58] In fact, MMR factors form large complexes with many repair factors, illustrated by

the BRCA1-associated genome surveillance complex (BASC).[59] Importantly, clonal evolution is induced in conjunction with MSI and the associated mutagenesis[6]; MSI induction is accompanied by a very high rate of mutations, even compared to the mutation level induced during canonical replication under MMR deficiency.

4 | GENOMIC DESTABILIZATION AND ASSOCIATED MUTAGENESIS

4.1 | Genomic destabilization-associated mutagenesis

Genomic destabilization is associated with an increased risk of mutagenesis, which results in the clonal evolution of cells with abrogated defense systems.[6] In fact, many base-substitution mutations in cancer cells are likely to be induced in association with genomic destabilization. A typical example is kataegis; that is, localized hypermutation occurring in a small genomic region that is specifically induced in close proximity to genomic rearrangements.[60,61] Further supporting this argument, mutations in cancer cells are more likely to accumulate in heterochromatin regions, where chromosomal translocations occur at high frequencies.[62-64] Furthermore, a recent in vitro model study revealed that the mutation rate in the growing cellular state is limited even in MMR-deficient cells that cannot repair replication errors. By contrast, the mutation rate is highly elevated during genomic destabilization.[6]

A longstanding question is how mutations that induce clonal evolution are caused. Genomic destabilization-associated mutagenesis is probably the major cause of the resultant clonal evolution. In fact, clonal evolution of cells with mutations in the ARF/p53 module is triggered by genomic destabilization, at least in an in vitro model.[6]

4.2 | Genomic destabilization-associated induction of cancer-driver mutations

Besides base-substitution mutations, many mutation types can be induced in association with genomic destabilization. This includes chromosomal deletions and translocations, which are inducible by genomic destabilization but not polymerase errors (Figure 3A,B).[35,36] For example, the cancer suppressor *Cdkn2a* is often inactivated by deletions.[40,65-67] In addition, some types of cancer drivers are generated by gene fusions caused by chromosomal translocations.[68,69]

Loss of heterozygosity also occurs during genomic destabilization and leads to dysfunction of cancer-suppressor genes (Figure 3C).[38-40] The probability of mutations in both alleles of a gene due to random replication errors or erroneous repair is extremely low. By contrast, LOH could increase the probability of biallelic mutation during genomic destabilization. After one allele is mutated, the second mutation can be induced in a "copying and pasting" manner by erroneous HR between the allelic loci. In this scenario, the probability of mutation propagation is as high as 50%.[38,70] Indeed, LOH is widely observed in cancer cells and results in the dysfunction of cancer-suppressor genes.[71]

The rate of base substitutions is also elevated during genomic destabilization (Figure 3D). Replication stress-associated DSB are primarily targeted by HR, in which high-fidelity replicative polymerases do not operate; instead, low-fidelity translesion synthesis (TLS) polymerases with no proofreading activity are induced.[6,72,73] Indeed, the mutation rate is highly elevated during genomic destabilization.[6] The induction of hypermutation during DSB repair is similar to somatic hypermutation of immunoglobulin loci, in which mutations other than those caused by deamination are induced by errors of the low-fidelity TLS Polζ during class-switch recombination.[74,75] Therefore, the rate of base-substitution mutations is also highly elevated when cells are subjected to genomic rearrangements.

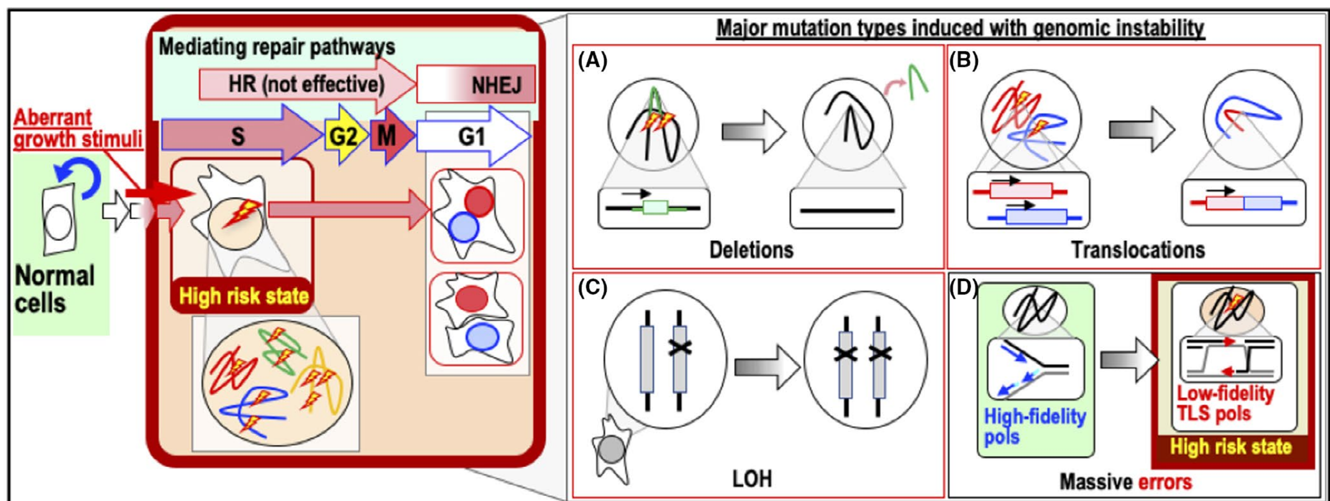

FIGURE 3 Genomic destabilization-associated mutagenesis. High levels of mutations are induced during genomic destabilization. Mutation types include chromosomal deletions (A) and translocations (B), loss of heterozygosity (LOH) (C), and massive base-substitution errors, which are induced due to the operation of low-fidelity translesion synthesis (TLS) polymerases during DNA double-strand breaks (DSB) repair (D)

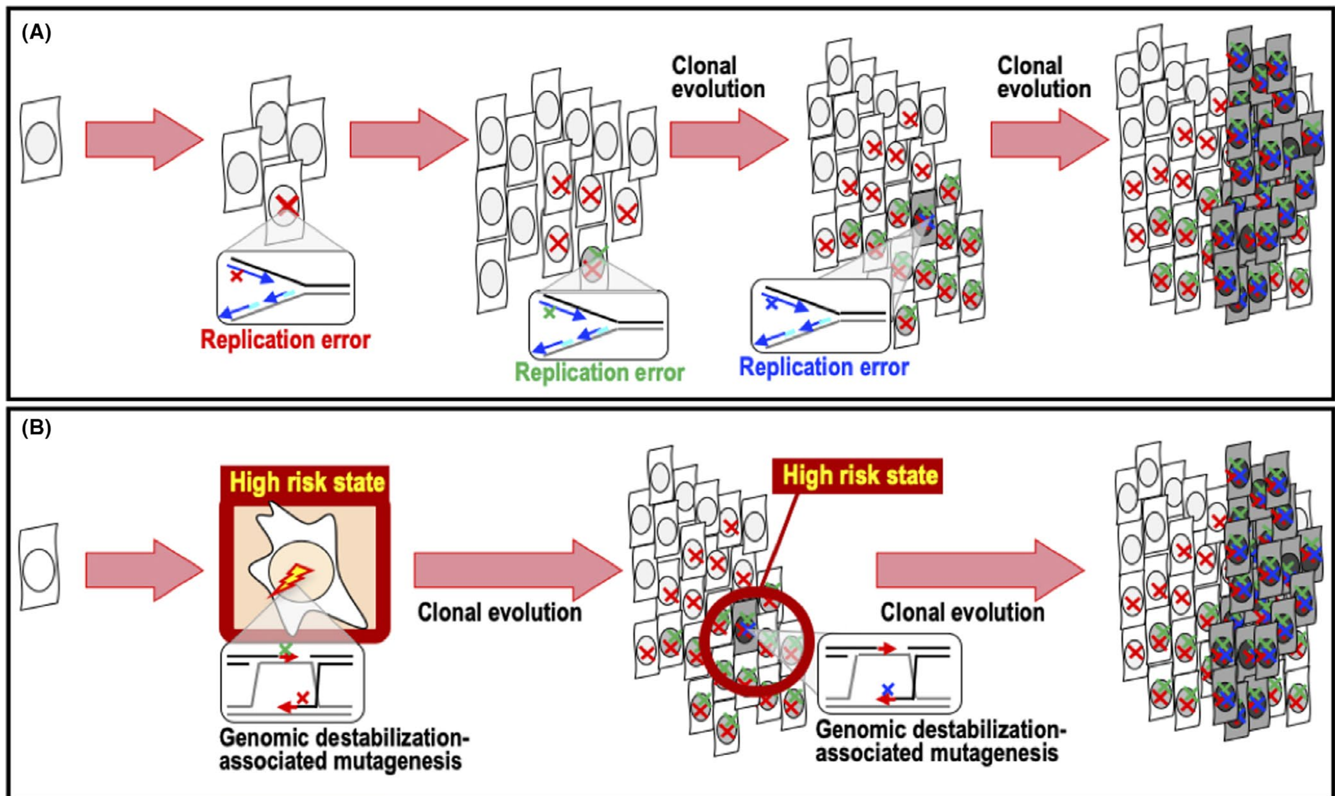

FIGURE 4 Two models of clonal evolution and cancer development. (A) Clonal evolution induced by replication errors. (B) Clonal evolution triggered by genomic destabilization

4.3 | Differences in the spectrum of ARF and p53 mutations between chromosomal instability and microsatellite instability induction backgrounds

It is well established that ARF/p53 mutations are generally a prerequisite for cancer development. Intriguingly, while p53 mutations and deletions are often seen in CIN-associated cancer,[76] they are uncommon in MSI-associated cancer, in which ARF is usually dysfunctional.[77] This suggests that ARF and p53 mutation spectrum may differ between CIN and MSI cell backgrounds. However, the mechanism to cause the different mutation spectrum is still an open question.

5 | CANCER RISK

Another important question is whether mutations that promote cancer development are avoidable. It was once thought that most cancers are unavoidable because most mutations in cancer-driver genes are caused by random replication errors in cancer-driver genes (Figure 4A).[78,79] The standard view was that cancer mutations can be categorized into three types (ie, hereditary, replicative, and environmental). The environmental mutations are avoidable but the others, including mutations randomly induced during replication, are not.[78,79] Accordingly, secondary prevention is a major priority in efforts to prevent cancer-associated death.

Based on recent findings, however, the above hypothesis is probably untrue. Replicative mutations can be separated into two types: mutations randomly induced as replication errors during active growth (Figure 4A) and genomic destabilization-associated mutations induced by replication stress in cells expressing senescent cellular phenotypes (Figure 4B).[6] The latter is the major type and is closely correlated with the resultant clonal evolution (Figure 4B). Importantly, unlike the former, the latter is theoretically avoidable through maintenance of genome stability. In fact, a recent study revealed that genome stability can be maintained by consumption of certain types of polyphenols that exert a cancer-suppressive effect[80]; however, the mechanism underlying these compounds' effects on maintenance of genome stability remains unclear. Given that most cancers develop with genomic instability,[1,5] a cancer-prevention strategy involving maintenance of genome stability might be applicable to many cancers, suggesting a future direction for cancer-prevention research.

DISCLOSURE

The authors have no conflicts of interest to declare.

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