Chapter

Genomic Instability and DNA Repair in Cancer

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Abstract

Mutations in genome are essential for evolution but if the frequency of mutation increases it can evince to be detrimental, for a steady maintenance there exist a detailed complex system of surveillance and repair of DNA defects. Therefore, fault in DNA repair processes raises the probability of genomic instability and cancer in organisms. Genome instability encompasses various aspects of mutations from indels to various somatic variants. The chapter tries to present an overview of how cancer puts up several ways to ensure suppression of the fidelity in our DNA repair system. Cancer cells assure failure of efficient DNA repair mechanisms by innumerous ways, by mutation and epigenetic modifications in repair genes themselves or genes controlling their expression and functions, other by some catastrophic events like kataegis, chromothripsis and chromoplexy. These are clustered mutations taking place at a particular genomic locus which deluge the repair process. Cancer generation and evolution is dependent largely on genome instability, so it applies many strategies to overcome one of its basic obstacles that is DNA repair, targeting these DNA repair genes has also demonstrated to be helpful in cancer therapy; but an intricate understanding of recalcitrant process and mechanisms of drug resistant in cancer will further enhance the potential in them.

Keywords: genome instability, DNA repair, cancer, epigenetic modifications, clustered mutation

1. Introduction

Genome is the basis of life of an organism and mutation in genome is essential for adaptation [1]. A mutation is a change in genomic sequence, they are the result of mistakes a cell makes while copying a piece of genome during replication or sometimes mutation is influenced by exogenous agents. Mutations have the capacity to influence gene expression depending on their location in the genome, gene structure and intergenic region. They possess this power to affect with such consequences because mutation in coding region of the genome might give rise to a truncated protein with no use or might compromise its fidelity. Alternatively, it can also endow the protein with some advantages with its function. Their presence in the regulatory region may increase or decrease its expression. This change in level of expression also affects cellular mechanisms since proteins are required by the cell in specific amounts. Therefore, these changes give either an advantage or a disadvantage depending on the effect it may produce but with a higher rate of mutation cell loses

its capacity to maintain genome integrity, that give rise to genome instability, it is a range of DNA alterations which irreversibly change information content of the genome. To keep a check on all such mutational process cell has an elaborated system of DNA repair and checkpoints [2, 3].

In unicellular organisms, a delicate balance exists between maintenance of genome stability and the tolerance to genome instability. They have harnessed this instability to mediate phase and antigenic variation that instead imparts them advantage for survival [4]. But any catastrophic changes in the genome are detrimental to them, in simple words genome instability for unicellular organisms is deleterious if it becomes impossible for them to take a control on it. For complex multicellular organisms like humans, we accumulate DNA damage over years that lead to genome instability and this genome instability is one such reason for aging [5]. A lot of studies are available that clearly state that the genome instability is a hallmark of aging and cancer. Cancer as we all know is a disease where the cell's own regulatory system goes wrong and there is uncontrolled division and to relief itself from cell cycle checkpoints and escape immune surveillance and apoptosis a cell must accumulate enough mutations. Cancer usually arises from benign tumor; these are localized abnormal growth of cells that cannot spread to other parts of the body. They undergo some more mutations and turn malignant now they have the capability to spread. Although there are certain types that do not form benign tumors such as leukemias, lymphomas and myelomas because of their nature [6].

There are several ways by which cell resist accumulation of DNA damage such as scavenging DNA damaging molecules, repairing erroneous DNA and at last if the cell is damaged beyond repair then apoptosis [7]. Despite all these measures the cell sometimes gathers enough mutation for the genome to be unstable and it to be cancerous [8]. Reasons behind DNA damage can be endogenous or exogenous. These damages are first perceived by the cell and a process of DNA repair is triggered on. Any discrepancies in the process of DNA repair predispose the cell to malignant transformation. Therefore, detection and repair of changes in the genome is prime to maintain cellular integrity. From this we can very well understand the importance of the role by DNA repair mechanism on maintaining genome integrity [9].

Its role can also be understood in hereditary cancers where most of the time one allele of a gene involved in one of the repair systems remains mutated at birth and the other turns mutated in course of time and the cancer arises. There are other example like the famous Breast Cancer genes; namely BRCA1 and BRCA2 where mutation in any one of them predisposes to breast and ovarian cancer. About 5–10% of people having mutation in BRCA genes encountered cancer once, mutation in these genes also predisposes the individual to cancer recurrence. We all know mutations owe their effectiveness to their space, therefore not all mutation in these genes is potentially effective in predisposing the person to cancer. Several mutations are identified till date and categorized by its influence. Both the genes BRCA1 and BRCA2 are involved in transcriptional regulation in response to DNA damage, most of these functions are mediated by the cellular proteins that interact with them [10]. BRCA1/2 is a tumor-suppressor and gets recruited in DNA damage loci, it has many other functions like damage induced cell cycle checkpoints activation, its association with homologous recombination as well as non-homologous recombination has also been established [11]. The exact mechanism behind BRCA controlling all these processes is still unclear but there is evidence of its direct association [10]. There is a specific type of drug available acting on these cells with mutation in BRCA genes; poly ADP-ribose polymerase (PARP) inhibitors. Their effect is specific to the cancer cells as they are usually deficit in homologous recombination, we will discuss this later in the chapter [11]. Current chapter gives a description on mutations and epigenetic modifications of the DNA repair genes which aid genomic instability in cancer.

2. An introduction to genome instability in cancer

Genomic instability refers to chromosomal changes, ploidy change or changes in nucleic acid sequence. In multicellular organisms, genome instability is fundamental to carcinogenesis [12]. Genome instability in cancer is associated with the ploidy change, chromosomal translocations, inversions, insertion, deletions, DNA breaks or any abnormal changes in DNA tertiary structure that can cause DNA damage, or the misexpression of genes (**Figure 1**). Ploidy change is accumulation of extra copies of chromosome(s) or parts of chromosome. Chromosomal translocations are phenomenon when a part of the chromosome breaks and get attached to some other chromosome. Inversions are breakage of a piece of DNA and getting attached to the same position in an inverted orientation. It happens when the DNA undergoes breakage and rearrangement. Insertion as the name suggests is the insertion of nucleotides on a locus in the genome. In deletions, there is deletion of nucleotide from specific loci in the genome. DNA breaks are either double stranded or single stranded depending, single stranded are easily repaired because here template is readily available but there are specific repair pathways dedicated to double stranded breaks since template is lost and the repair pathways has to bring it from the other pair and in some cases, there is error prone repair [8, 13]. Microsatellite instability is another such phenomenon influencing genomic instability. Genome instability fuels the cancer cells with changes that help it to evolve and escape death. Additionally, it also plays a critical role in cancer initiation and progression by overcoming immune surveillance, attaining uncontrolled cell division, more DNA damage, etc. [14]

In normal cells, genome is protected at every stage of the cell cycle, every step from DNA replication to chromosome packaging. Every step is very precisely monitored for faults. Several processes are involved in this such as cell cycle checkpoints, DNA damage checkpoints and DNA repair. When a normal cell turns cancerous, these fault monitoring systems are manipulated; presence of genomic instability itself indicates the failure of one or many of these safety nets (**Figure 1**) [13]. Interpreting the underlying mechanisms for imbalance in genome integrity would yield new avenues for precision therapies and clinical decision-making. Lot of research has been centered to genome instability to understand it and hold control over its initiation and progress in a hope to conquer cancer, which is the world

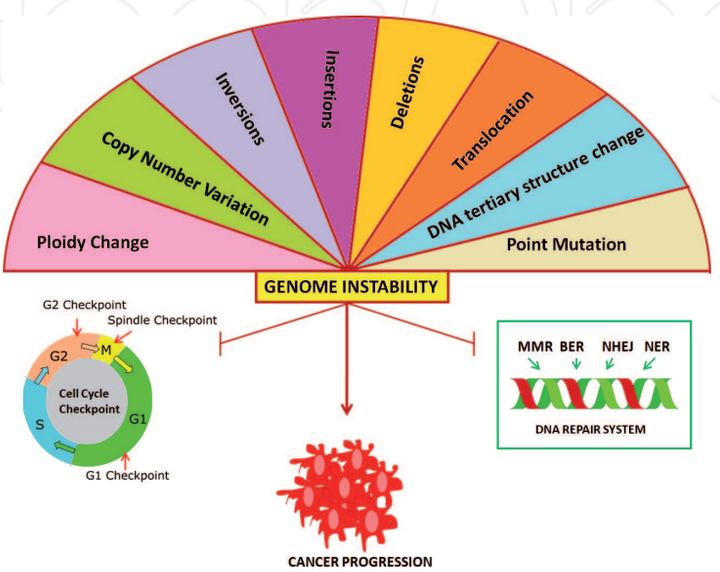

Figure 1.
Genomic instability influencing cancer.

leading cause of death. But still a clear picture of its origin or prevalence is far from our understanding [15].

All the mutations which are results of genomic instability are not hazardous, some of them just exist without much influential function. The ones with potent negative effects are termed as driver mutations and the one without are termed as passenger mutations. Even passenger mutations contribute to tumorigenesis but has less influence. Diver mutations are usually the mutations that are present in tumor suppressors, DNA repair genes, etc. A lot of studies are done to understand these driver mutations but again there is no clear evidence [15].

In hereditary cancers, genomic instability arises from mutations in DNA repair genes that drives cancer development, as predicted by the mutator hypothesis. On the other hand, sporadic cancer, the molecular basis of genomic instability remains unclear, but recent high-throughput sequencing studies suggest that mutations in DNA repair genes is one of the major mechanisms of inducing genomic instability. Still there remains a debate on either tumor suppressor genes or the DNA repair genes are the major source for genome instability in cancer, but it is established that DNA repair mechanisms is one of the prime targets [12].

3. Influence of cancer on DNA repair pathways

Source of mutation in DNA of normal cell are either from faults in DNA replication procedure or from carcinogens. The fidelity of eukaryotic DNA polymerase is very high with about 10^{-10} mutation per 1000 nucleotides, yet sometimes it miss, and mutation occur. Carcinogens encompasses all the factors that cause DNA damage and evoke carcinogenesis, they are such as ionizing radiations, UV radiations; some non -radiating ones are such as alcohol, tobacco, cigarette smoke and such other abusive products. These products include reactive oxygen species, deaminating agents, alkylating agents, polycyclic aromatic hydrocarbons, base analogs, intercalating agents, etc. [16]. Not only these, but there are also some microbes such as *Helicobacter pylori* and Human papilloma virus which induce inflammation leading to generation of various reactive oxygen species and generation of cancer. But still till date a specific reason for cancer cause has not been elucidated [17]. Several theories describe cancer cause but a proper picture of its causal and turning deadly is not yet established. It is very clear that cancer is not a one step process while it is complex, associated with several factors coming together to generate a system for freeing itself from crunches of checkpoints for regulating cell division. In normal cells these mutations are checked on by DNA damage checkpoints or cell cycle checkpoint, these are a series of biochemical pathways that are in constant surveillance to ensure integrity of the genome upon encountering any defects, they halt cell cycle progression and activate DNA damage repair mechanisms, then ensure repair of the faults and resume cell cycle progression [18] as shown in **Figure 2**.

There are several type of repair pathways namely: nucleotide excision repair (NER), base excision repair (BER), DNA mismatch repair (MMR) and double strand break repair which includes homologous recombination (HR) and non-homologous end joining (NHEJ). Every one of them are specialized in a specific type of repair pathway [4]. A detailed description of them with the mutation and modifications that are present in genes involved in these pathways in cancer cells can give us much understanding of exploitation of the process by cancer.

Nucleotide excision repair (NER) repairs bulky lesions and large adducts that distorts double stranded helix under conditions when only one of the two DNA strands is affected, UV radiation and chemical mutagens include such damage. These mutagens cross-link adjacent pyrimidine bases and purine bases and creates

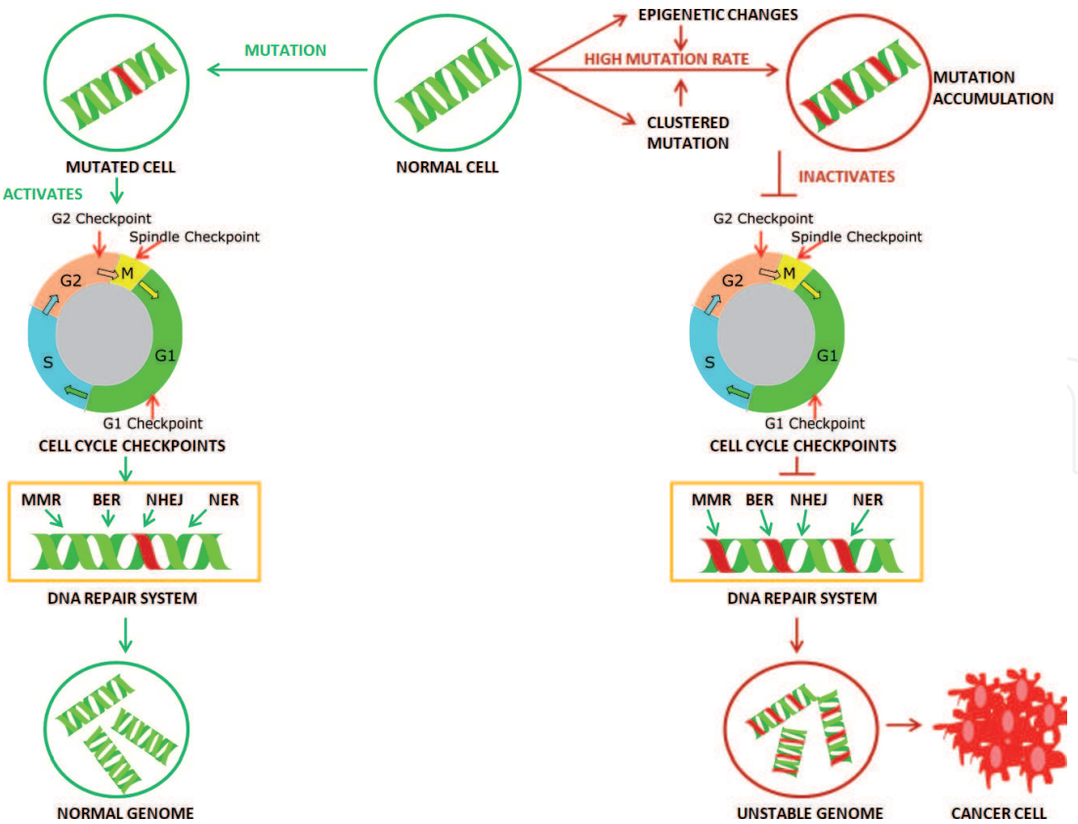

Figure 2.
Schematic illustration of relationship between mutations, DNA repair system and cancer.

intra-strand adducts, such damage blocks basic cellular systems like DNA replication and transcription [19]. NER can adapt to many structurally unrelated types of damage, to perform its diverse work, NER employs two sub pathways: global genome repair (GG-NER) and transcription coupled repair (TC-NER). The sub pathway names hint their distinctive roles, transcription coupled repair act on lesions that block progression of an active transcription site, a locus in the genome where an RNA polymerase is actively functioning and global genome repair is active in all phases of the cell cycle, repairing damages [20]. NER first recognize the damage, gather its associated complex and unwinds that specific region of DNA locus, makes incision on both points, remove the damage, synthesizes new nucleotides using other DNA strand as template strand and ligates the DNA [21]. Several different proteins are involved in the process; a nine-unit complex called transcription factor IIH (TFIIH) executes the first phase of repair, this complex includes two helicases, XPB and XPD and two other proteins, XPA and RPA that open the helix. It remains attached to the DNA while two different endonucleases, XPG and XPF, the latter acting in conjunction with ERCC1 perform precise cutting on one side of the damaged strand, several nucleotides away from the damage. After this initial step, RPA mediates the assembly of a second repair complex where Replication factor C (RFC) binds to excision region and facilitates PCNA that binds to DNA polymerases δ and ε, preventing them from falling off the strand before the reconstruction has been done and ultimately Ligase I attaches new repaired strand to the pre-existing ones [22].

The base excision repair (BER) pathway corrects damage occurring from oxidation, alkylation, deamination, and ionizing radiation, these lesions produce mild damage involving few bases the mutation usually cause base mispairings [23]. In this case mutagens are usually endogenous; here by the term mutagens we mean mutation causing agents, here only the damaged nucleoside is removed by cleaving

its *N*-glycosidic bond, leaving an abasic (AP) site [24]. One of BER's exclusivity depends on its 11 damage-sensing glycosylases, these glycosylases remain bound to the site to ensure that the fragile AP site never remains unattended, APE1/Ref1, or AP endonuclease/Redox Factor 1, process the loose ends creating special termini to accept the new base [25]. XRCC1 stabilizes the damaged area and coordinates sequential binding and release. XRCC1 also acts as a helicase, the process then includes a sliding clamp PCNA (proliferating cell nuclear antigen) and an additional stabilizer that also inserts the newly synthesized nucleotides, replication factor-C, or RFC [26].

During DNA replication, proofreading polymerases sometimes may fail to detect errors made by DNA polymerase. Mismatched repair (MMR) in a post-replicative repair mechanism steps in this scenario to clear the errors. MMR's damage sensors can differentiate between the parental DNA strand and the newly synthesized DNA strand and only remove the mismatch in newly synthesized strand, using parental strand as a template, MMR remove the mismatch and synthesizes new DNA strand [27]. MMR corrects single-base mismatches and small insertion/deletion loops by one of the two damage recognition complexes MSH2:MSH6 or MSH2:MSH3, MSH2:MSH6 recognizes single-base substitutions and the smallest insertion/deletion loop and MSH2:MSH3 insertion/deletion loop involving up to 10 nucleotides [28]. Then they recruits a complex, comprising MutL homolog 1 (MLH1) and its binding partners, post-meiotic segregation increased protein 1or 2 (PMS1 or PMS2), MSH and MLH complexes form sliding clamp that moves along the DNA sequence till it encounters a single-strand DNA gap, replication Protein A (RPA) functions as indication at the damage site. Then MutL complex encounters the cluster, it allows a DNA exonuclease (Exo1) to enter the DNA structure, guided by the MLH:MSH complex, Exo1 removes the damage portion plus some more nucleotides and a DNA polymerase, Pol δ synthesizes DNA in those loci of excision. Finally, Ligase I joins the new DNA to the existing daughter strand [29].

Double-strand breaks (DSBs) are the most serious, hazardous, very complex to repair, they are rare but there are instances of its occurrences; most common ones are due to breaks in replication forks when polymerases stall at the site of unrepaired base lesions and by exogenous agents like ionizing radiations, etc. Anticancer treatments comprising of chemotherapeutics and radiation therapy can also induce multiple kinds of double-stranded DNA damage [30].

DSB repair faces many challenges such as loss of physical integrity on both strands due to which there is loss of information, like when one stand is damaged one can retrieve information from the other strand but if there is a double strand break there is no template for synthesis of the new strand, to repair such damage, human cells employ two main pathways: nonhomologous end joining (NHEJ) and homologous recombination repair (HR). Cell cycle checkpoints evaluate end processing required, which partially dictates how DSBs would be repaired [31]. NHEJ operate during any cell cycle phase but is most active in G0 and G1 (before DNA replication), whereas HR is active during S and G2 phases (after replication), its prone to error whereas HR is template based therefore its fidelity is higher and this makes the process a little complicated. Damage sensor complex is common to both NHEJ and HRR is Mre11-Rad50-Nbs1 (MRN). MRN's functionality is only one of many mysteries of DSB repair, there are still a lot to be discovered regarding HR and NHEJ. The following paragraphs summarize the main HR and NHEJ pathways [22, 32].

Nonhomologous end joining (NHEJ) rejoins DSB ends without a template, it does not search for or use a large segment of DNA for determining which bases were present before the damage occurred and search which is the other end of the breakage. Therefore, repair proceed quickly with the potential for loss of nucleotides from either side of the DSB junction, alteration of base pair sequences at

the breakpoint or getting attached to some other end that does not belong to the sequence in case of multiple breaks. Thus, NHEJ can contribute to a large amount of mutation but it is preferred by the cell [33]. The main challenge of NHEJ is to collect the two free ends into immediate proximity and protect them from nucleolytic attack. The Ku heterodimer, a damage sensor and a lyase imparts protection and recruit other proteins for end processing. Then DNA-dependent protein kinase catalytic subunit (DNA-PKcs) binds to Ku and becomes DNA-PK, a docking port for various kinds of DNA end processing enzyme. Another complex XRCC4 + Ligase IV + XLF create a filament to bridge the ends. Ligase IV ligate across gaps and join processed DNA ends. Many aspects of NHEJ still remain a mystery, including whether its steps are sequential, iterative or flexible according to the complexity of the damage. The most studied form of NHEJ is V(D)J recombination, which occurs only in T and B cells and is essential for their development, maturation and generating lymphocyte diversity [34].

Homologous recombination repair (HR) is a complex template directed DSB repair mechanism, it gets activated after DNA is copied in S phase but before it divides in M phase, so that the two strands are still together held by cohesion complex and HR takes advantage of the other full copy of adjacent DNA. This enables HR to find a large area of homology on the sister chromatid and use it as a template to reconstruct the damaged DNA strand [35]. HR plays significant role in maintaining genomic stability, in the basic step, MRN forms single-stranded DNA at the DSB end, ssDNA extends beyond the original breakpoint enabling Rad51 to attach to 3′ end, RPA binds to the naked stretch of DNA so that Rad51 can sit on the ssDNA and find DNA sequences like the 3′ overhang. When Rad51 encounters the locus with homology it invades the double strand, creating a DNA heteroduplex. Rad51 facilitates exchange of homologous DNA sequences within the sister chromatid. The overhang progressively extends as new nucleotides are generated beyond the original breakpoint, Nbs1 recruits other repair proteins to the site, Rad50 serves as a tether; MRE11 possesses both exo- and endonuclease functions [35]. During synthesis, as the loop is pushed, an X-shaped structure develops, called a Holliday junction, at the end this Holliday junction is resolved and ends are ligated Many HR genes like BRCA1, BRCA2 are involved in genomic instability generation and cancer [36]. Above mentioned facts have lightened up the understanding that cell have a very elaborately designed DNA repair system for damages now let us see how cancer wangle it through various measures.

4. Mutations in DNA repair genes in cancer

DNA, the genetic component of a cell, often gets damaged when exposed to any endogenous or exogenous agent like radiation, smoke, macrophages, ROS, etc. Different DNA repair pathways like base repair, mismatch excision repair, homologous recombination etc. help in repairing these DNA damages. But the expression of genes involved in these repair pathways sometimes gets reduced due to germline mutation, epigenetic alterations, somatic mutation, etc. As a result, the unrepaired DNA damage accumulates in cells. This accumulation might lead to further increase in epigenetic or somatic alteration, which helps in multiplying the altered field defects as well as different driver mutations that ultimately helps in the progression of cancer [22]. Details of few alterations affecting DNA repair genes are mentioned below.

4.1 Germline mutation associated with DNA repair pathways

Germline mutations of DNA repair pathways usually results in predisposing ones to cancer or having it by birth itself if abnormal gene is inherited from one

of the parents and other gene copy gets inactivated in a somatic cell later in life or both copies of a gene can get deactivated. Ultimately, that results in loss of heterozygosity which leads to a deficient response of DNA repair genes. Such affect from mutations are also seen if it is present in tumor suppressor cells [37].

Genes involved in the mismatch repair like MLH1, MSH6, MHS1 are often associated with Lynch syndrome, hereditary non-polyposis colorectal cancer (HNPCC), is the most common cause of hereditary colorectal (colon) cancer when undergoes monoallelic mutation but are associated with Constitutional mismatch repair deficiency syndrome due to biallelic mutation across children and adolescent [38].

Genes involved in homologous recombination like BRCA1/2 and BRIP1 are associated with hereditary breast and ovarian cancer syndrome when undergoes biallelic mutation. Defect in these two genes often results in breast cancer [38].

4.2 Somatic mutational signatures are associated with DNA repair pathways in cancer

Somatic mutations are basic to cancer and finding their occurrence on DNA repair genes is expected, researchers have well-established effect and consequences from a few of them. The substitution mutation signatures with homologous recombination genes like BRCA leads to homologous recombination failure in breast cancer, other than that promoter methylation also contributes to BRCA gene defects in different tumors [38]. Apart from substitution, rearrangement signatures are also involved in BRCA of breast cancer, these signatures include tandem duplication, inversion, deletion, translocation, etc. For example, tandem duplication is associated with the BRCA1 mutation, whereas small deletions are associated with BRCA1/2 inactivation, indel rearrangement signature is associated with BRCA deficiency [39].

Unlike homologous recombination repair, substitution mutation signatures are also associated with the Mismatch excision repair system. For example, high rates of substitution and indels are seen in C.G to T.A transition at NpCpG sequences. Also, rearrangement signatures are associated with MMR genes, which are often found in breast cancer [39].

High expression of *XRCC-1,* interacts with DNA ligase III, polymerase beta and poly (ADP-ribose) polymerase is associated with early tumor stage in oral squamous cell carcinoma. Accumulation of single strand breaks downregulates protein APE1 responsible for DNA incision during BER helping the conversion of single strand breaks to double stranded ones [40].

Overexpression of *ERCC1* in prostate cancer has association with the formation of chromosome aberrations. It is shown to inhibit apoptosis in esophageal squamous cell carcinoma. Some of its polymorphisms also indicates prognostic markers [41].

5. Epigenetic alterations associated with DNA repair pathways

Likewise mentioned earlier cancer applies several methods to take control over repair mechanism, epigenetic alteration is another such technique, here the DNA sequence remains intact, but expression and activity of the gene is affected. It's the technique due to which we have different type of cells in our body despite having the same genetic makeup and most importantly it's hereditary. Epigenetics include covalent modifications like methylation, ubiquitylation, sumoylation, phosphorylation to histones or DNA, sRNA, miRNA [41]. The following information

is on promoter methylation, miRNAs and chromosome remodeling by histone modification.

5.1 Epigenetic alterations by promoter methylation

One of the most common ways of epigenetic alteration is by promoter methylation, this is often regulated by cytosine methyl transferases, genes get inactivated by methylation in 5-carbon of cytosine of 5′-CpG-3′ dinucleotide sequence at either promoter regions. Two of the genes involved in base excision repair namely Methyl-CpG Binding Domain 4, DNA (MBD4) and Thymine-DNA glycosylase (TDG), both are glycosylases with same function of removing mismatches by hydrolyzing carbon-nitrogen linkage between the sugar and the phosphate backbone of DNA and mis paired thymine. Due to promoter methylation in these two genes, it is found that BER pathway often gets suppressed in cancers like colorectal, myeloma, ovarian, etc. [41].

The *XPC* gene which encodes a protein that is a key component of the XPC complex involved in GG-NER, promoter methylation of this gene often leads to NER function loss in cancers like bladder cancer. Other genes of the nucleotide excision repair pathway, RAD23A and ERCC1 genes also get inactivated by promoter methylation in different cancers like RAD23A in multiple myeloma cancers and ERCC1 in glioma cancer. Genes of the mismatch repair pathway namely MLH1, MSH2, MSH3, and MSH6 also gets suppressed by promoter methylation in various cancers like ovarian, gastric, etc. [41, 42].

Two genes BRCA1 and BRCA2 of homologous recombination system also have compromised activity by promoter methylation in different cancers like breast, gastric, uterine, etc. One of the genes, involved in non-homologous end-joining, XRCC5 encodes the heterodimer Ku (composed of K70/K80), which facilitates binding to nascent DNA breaks often gets epigenetically inactivated by promoter methylation is seen to be associated with cancers like adenocarcinomas [43].

Direct reversal of DNA damage is the most energy efficient repair system, but its capabilities encompasses only certain damage categories such as pyrimidine dimers formed by UV radiation, O6 adducts like alkyl groups on nucleotides from chemotherapy. *O*6-methylguanine-DNA methyltransferase (MGMT), catalyzes transfer of methyl groups on DNA to its own molecule, methylation on its promoter inactivates it like the other DNA repair genes, this is often associated with cancers like glioblastomas, colon cancer, lung cancer, lymphoma etc. [44].

5.2 Epigenetic alterations due to chromosome remodeling and histone modification

The miRNA is synthesized as primary non-coding RNA these are then processed into mature effective ones which can alter expression of its target genes. On those target genes it influences the methylation status in the promoters and we know that the methylation status of promoters are related to their expression levels or they can directly target epigenetic factors, such as DNA methyltransferases or histone deacetylases, regulating chromatin structure for altered expression. Some genes of mismatch excision repair like MLH1, MSH2, and MSH6 are inactivated by such process, by the action of miR-155 (**Table 1**) [45].

Low expression of miRNA-15 suppresses promoter activity of BRCA1 by recruiting an enhanceosome mediated by HMGA1 [46]. miRNA-16 influence transcriptional activation of HMGA2 protein that again suppress ERCC1, is required for the repair of DNA lesions such as those induced by UV light or formed by electrophilic compounds including cisplatin. HMGA (High Mobility Group proteins with AT

DNA Repair Pathways	DNA mutation (Germline)	DNA mutation (Somatic)	Epigenetic Changes	References
BER		*APE1,XRCC-1*	MBD4, TDG	[40, 41]
NER		*ERCC1*	XPC, RAD23A, ERCC1	[41, 42, 47]
MMR	MLH1, MSH6, MHS1	MSH2, MSH6	MLH1, MSH2, MSH3, MSH6	[38, 39, 45]
Homologous Recombination	BRCA1/2, BRIP1	BRCA1/2, PALB2	BRCA1, BRCA2, HMGA1	[38, 39, 43]
NHEJ			XRCC5	[43]
Direct Reversal DNA Damage			MGMT	[44]

Table 1.
DNA repair pathway genes affected in various ways in cancer.

hook) code for a chromatin-associated protein that can modulate transcription by altering the chromatin architecture, HMGA1 and HMGA2 are two of its types [47].

As mentioned above that cancer cells put up several techniques to ensure faulty DNA repair system in the cell. The faulty DNA repair system now provides cancer cells with ability to produce more and more mutation. This higher rate of mutation gives the cancer cell advantage to manipulate cell machinery for uncontrolled growth. After choreographing the regulation of DNA repair system cancer then effects its fidelity, for the purpose it brings into the picture clustered mutation, which is a specific characteristic of cancer and cancer cells owe it to faulty DNA repair systems. An elaborate analysis of the clustered somatic mutations can identify error-prone DNA repair mechanism as a common source of mutations in active chromatin in human tumors [48].

6. DNA repair and clustered mutation in cancer

Clustered mutations, as the term suggest is localized hypermutation. There are three of its types namely- chromoplexy, chromothripsis, kataegis.

Chromoplexy refers to a class of complex DNA rearrangement observed in active regions of the genomes of cancer cell. The mechanism underlying complex rearrangements has not been established. But a proposed model says in the process DNA is brought together by the transcription factor working in a co regulated manner on different genes [49]. The process makes DNA in those places vulnerable to breakage and malfunctioning of DNA repair system make a jumbled-up repair of those broken pieces. Although this model has not been established but it's taken into account because chromoplexy is prevalent in only areas where there is active transcription and it can explain how DNA from multiple chromosomes may participate in a single chromoplexy event [50].

Chromothripsis is another such mutational process in which a number of chromosomal rearrangementsoccur in localized genomic regions in one or a few chromosomes together. The process takes place in a single event, where in the genomic space arise several double strand breaks. These breaks are then again joined by DNA repair system in a non-homologous manner. Once again, the crucial role of DNA repair system in the process of cancer survival and evolution is entrenched [51].

Kataegis mutational clusters are several hundred base pairs long, alternating between a long range of C → T and G → A substitutional pattern. This says it takes place in one of the two template strands during replication. It is more common than chromoplexy and chromothripsis. Kataegis hypothesis includes mismatch repair to activate and repair on locations of mismatch, making those regions single stranded and these single stranded regions are substrate to various modifying enzymes. These modifying enzymes then promote formation of mutation clusters along the entire track of breakage [52].

7. Evidence of clustered mutation influencing repair pathways

APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) enzymes and translesional DNA synthesizing enzyme are found to be associated with these events. There is literature available on this context. APOBEC enzymes are cytidine deaminase that is responsible for C → T transitions [53, 54]. H3K36me3 chromatin is normally protected from such somatic mutations, it is tri-methylation at the 36th lysine residue of the histone H3 protein, it relishes this protection from somatic mutation because of increased activity in canonical mismatch repair machinery at its locations. However, exposure to some carcinogens results in increased activity of a non-canonical, error-prone, mismatch repair pathway involving (POLH) DNA polymerase eta, which results in a relative increased mutation rate in H3K36me3-marked regions. This explains that some factors act as carcinogens not because they increase the mutation rate but because they relocate mutations to the more important regions of the genome. These environmental factors include alcohol, ionizing radiations, UV radiations etc. [6].

There are other evidence stating clustered mutations are driven by break induced replication (BIR) like mechanisms, which is associated with homologous recombination. Tremendous progress in whole genome analysis revealed that BIR is likely the mechanism of multiple genomic rearrangements in humans that give clustered mutation. To the date, there is no clear understanding of how BIR transforms from a beneficial pathway aimed at rescuing cells into a dangerous mechanism with high destabilizing potential [55].

These events are very common to cancer cells. They serve as source for catastrophically higher rate of mutational events giving rise to sustainable amount of genomic instability. And as mentioned several times before genomic instability is the prime mechanism for the cancer cell to hold control over cellular machinery for uncontrolled division these events are very specific to cancer cells and a proper process of these events has not yet been elucidated [51]. But we can clearly see the potential role of DNA repair systems in these clustered mutational events. Through clustered mutations the cancer cell tries to exhaust DNA repair pathways. Repair pathways are meant to repair the DNA at a specific rate, and they are designed to tackle a limited burden. When mutation rate become overwhelming for them, their fidelity exhaust and that is the opportunity cancer cells create to accumulate mutation [53].

Cancer cells first changes the expression and regulation of the DNA repair systems by either epigenetic modifications, mutating its coding sequence or regulatory sequence. This in turn gives error prone DNA repair system for clustered mutation. Again, the clustered mutation also exhausts the DNA repair systems leaving no chance for fixing the genomic instability taking place in the cell. There still lies a debate on how these catastrophic mutational processes occur. But there is proof that they are indebted to faulty repair systems for their birth [54].

8. Cancer therapy targeting DNA repair pathways

Presently there are a few chemotherapeutic drugs and some of them are even in phase 2 or phase 3 trials. This itself emphasize the crucial role DNA repair pathways and proves that it is an important chemotherapeutic target. Basically, DNA repair inhibitors are used as chemotherapeutic drug they make already fragile DNA repair system of cancer to collapse leading to destruction of cellular homeostasis ultimately leading to cancer death [13, 26].

8.1 MGMT inhibition

The *O*6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein that removes alkyl group, was the target of the earliest attempt to develop a DNA repair inhibitor. MGMT is the most widely studied DNA repair mechanism [56].

In 1970s, nitrosoureas was introduced as a chemotherapeutic drug for glioblastoma and other malignant gliomas, it alkylates DNA at various positions on guanine, subsequently causing single- or double-strand damage which chemosensitiz cells to more damage by other drugs. Scientists quickly learned that something could reverse the DNA damage that they inflicted, that was MGMT. After some time, a potent MGMT inhibitor was used along with nitrosoureas but it did not work [26]. Although compromising MGMT fell short of expectations in chemo sensitizing tumors to alkylating agents, it was continued to be studied. There was evidence that in different cancer it is manipulated in different ways. But still there is not any effective drug involving this [57].

8.2 PARP inhibitors

The PARP is a nucleus specific enzyme that detects single strand breaks that are being formed spontaneously or during BER and binds to that position on the DNA strand. It then undergoes a structural change and begin synthesizing a polymeric adenosine diphosphate ribose (poly ADP-ribose) chain, which acts as signal for the other DNA-repairing enzymes. Three members of that family have roles in DNA repair, with PARP1 being the most important. It took a lot of time for PARP to be recognized as target for chemotherapeutic drug. First PARP inhibitor (PARPi) entered clinical trials, as a chemosensitizer like MGMT inhibitors. But its capacity as single agents to treat BRCA-deficient cell lines from germline breast cancers proved later. Olaparib was the first PARP inhibitor for ovarian cancer. Today, there are number of PARP inhibitors in clinical trials for not only breast cancer but also for other cancer types [58]. PARP's clinical efficacy on BRCA-deficient tumors is one of the most effective drug findings. PARPi function includes binding to PARP and inhibiting its function until next round of DNA replication, then accumulation of unrepaired SSBs will automatically get converted to DSB. Cells that are missing both alleles of BRCA 1, BRCA2 or PALB2 have no efficient HR functionality, which leaves repairs in the hands of NHEJ, its limited ability to repair extensive DSB damage leads to tumor cell death specifically because the cells with non-compromised HR can tackle these breaks very easily (**Figure 3**) [26]. That is why these are used as add on for effective cancer treatment. However, the effect of PARP inhibition is not as simple as it seems, there is lot more complexity to it like PARP's interactions with other proteins and PARP trapping [59].

PARP not only works with BER, but it also activates XRCC in HR pathway and is involved in a regulatory feedback loop with BRCA1. It also appears to inhibit the NHEJ pathway by inactivating DNA-PKcs and ATM's checkpoint activity. Moreover, it has a role in inflammation that proves its involvement in transcriptional regulation and many other biological functions associated to cancer. As mentioned earlier,

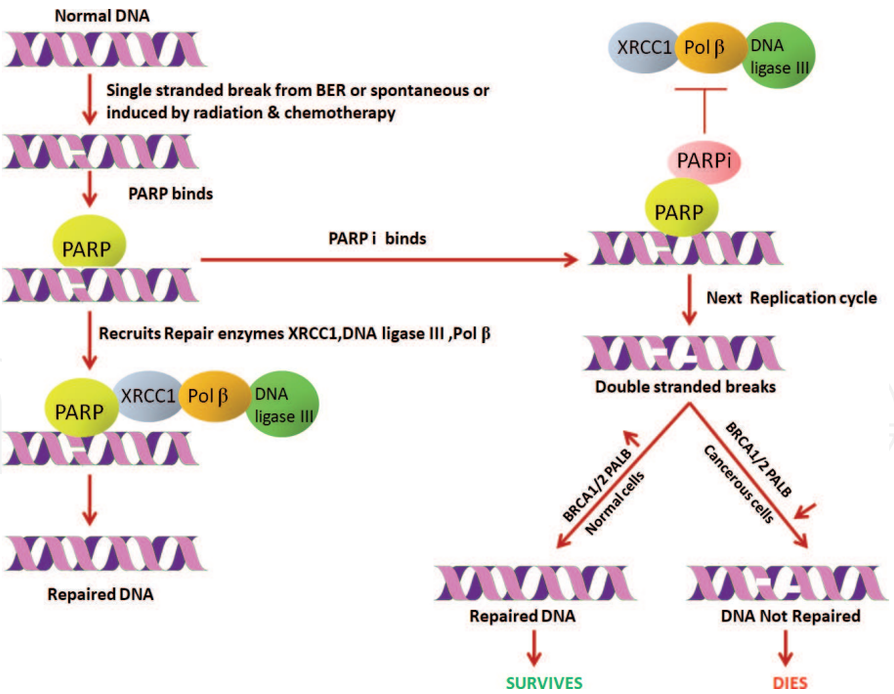

Figure 3.
Mechanism of action of PARP inhibitors.

cancers are notoriously clever when it comes to combat their survival, then they come up with new methods to the stress imparted to it by PARP inhibitors. Till date a lot of instances have been proved such as reverse mutation in BRCA, various ways of manipulating NHEJ, etc. [60].

9. Conclusion

Defects in the repair system assure genomic instability, this fuels disorderliness required for cancer to survive, sustain and evolve; that is why hereditary deficiencies in them makes the individual more susceptible to cancer. There are only some repair genes known to be exploited by cancer, a more extensive search of potential points might give a perspicuous picture. Researchers has been into understanding and finding cure to cancer since decades; but still till date we do not have a conclusion. This refers to its multiple techniques, different hierarchical steps and several process that it applies for its successful survival. DNA repair system is one of its basic targets, so cancer wangle it very well to establish its existence. It applies different mechanism from simple mutations to clustered mutations to various epigenetic changes just to assure a compromised repair system. A very elaborate venture of these changes can give us insight into generation of genomic instability by suppressing DNA repair in cancer. This information can help us get the much-sought effective treatment. Therapies targeting DNA repair genes already available are example to this.

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Conflict of interest

The authors declare no conflict of interest.

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