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Chapter

Recent Perspectives in Radiation-Mediated DNA Damage and Repair: Role of NHEJ and Alternative Pathways

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Abstract

Radiation is one of the causative agents for the induction of DNA damage in biological systems. There is various possibility of radiation exposure that might be natural, man-made, intentional, or non-intentional. Published literature indicates that radiation mediated cell death is primarily due to DNA damage that could be a single-strand break, double-strand breaks, base modification, DNA protein cross-links. The double-strand breaks are lethal damage due to the breakage of both strands of DNA. Mammalian cells are equipped with strong DNA repair pathways that cover all types of DNA damage. One of the predominant pathways that operate DNA repair is a non-homologous end-joining pathway (NHEJ) that has various integrated molecules that sense, detect, mediate, and repair the double-strand breaks. Even after a well-coordinated mechanism, there is a strong possibility of mutation due to the flexible nature in joining the DNA strands. There are alternatives to NHEJ pathways that can repair DNA damage. These pathways are alternative NHEJ pathways and single-strand annealing pathways that also displayed a role in DNA repair. These pathways are not studied extensively, and many reports are showing the relevance of these pathways in human diseases. The chapter will very briefly cover the radiation, DNA repair, and Alternative repair pathways in the mammalian system. The chapter will help the readers to understand the basic and applied knowledge of radiation mediated DNA damage and its repair in the context of extensively studied NHEJ pathways and unexplored alternative NHEJ pathways.

Keywords: Radiation, DNA damage, DNA repair, NHEJ, Alternative NHEJ

1. Introduction

Radiation is a natural part of our surroundings. Humans get exposure to natural radiation such as cosmic rays and radioactivity from earth and food. The diversified use of radiation in several technological procedures like power generation, sterilization of food products, industrial activities, therapeutics (radiotherapy), diagnosis,

nuclear weapon development etc., has increased the risk of exposure. Inadvertent accidents from nuclear power plant installations, nuclear weapon testing and illegal use of radioactive material in dirty bomb have raised an international concern for radiation safety [1–3].

Radiation therapy is the most common and deliberate exposure of high energy rays to living organisms. This exposure is mainly therapeutic for treatment of cancer but since there is no clear demarcation to protect the adjacent noncancerous cells leads to disastrous effect. The immediate exposure of high energy beam of radiation leads to destruction of cancerous cells. Whereas the adjacent normal cells are however exposed to these rays suffer adverse effects. It indirectly generates reactive oxygen species (ROS) inside the cellular system through hydrolysis of water. ROS directly targets cellular DNA and affect the cell survival by damaging macromolecules like lipid, proteins and carbohydrate. The damage induction in DNA molecules could be of various types like double strand break, single strand break, dimer formation, alteration of bases etc. Mammalian cells are equipped with very efficient DNA repair mechanism to handle these different damages [4]. Moreover among all, double strand breaks are known to be lethal damage for the cells. There are mainly two repair mechanisms that operate for repair of double strand breaks. These are 1) Homologous repair pathway (HR) 2) Non homologous end joining pathway (NHEJ). The basis on which cell decides to choose one of the two available pathways is simply on cell cycle phase, its type and damage threshold [5]. There are also exists third repair pathway i.e. Alternative non homologous end joining pathway (A-NHEJ) it is much slower than the above mentioned pathway. It comes into play when above mentioned pathway fail to repair the damage thus acting as a backup pathway. The presence of this alternative pathway has not been studied extensively but it has been speculated for its role in combinational cancer therapeutics. In this chapter, we have briefly described the various kind of DNA damage generated by radiation and role of DNA repair pathways specially NHEJ and A-NHEJ in handling the repair and their applications in progression of disease [6].

Radiation, which has particles with enough energy to rip electron from atoms or molecules is known as ionizing radiation. Radiation is the emission and propagation of energy in the form of rays or waves. The term radiation comes after the discovery of X-ray in 1895 by Wilhelm Conrad Roentgen. Henri Becquerel and Marie Curie have made significant contributions in studying the effect and application of radiation in various fields. Excitation and ionization properties are common responsible factor for radiation emitted by any radioisotopes. It has two major types: ionizing and non-ionizing radiation [6, 7].

1.1 Non-ionizing radiation

It does not carry enough energy to remove electrons from an atom or molecule. Because of their low energy, non-ionizing radiation poses a lower risk than ionizing radiation. Visible light, near ultra violet, infrared, microwave and radio waves are examples of non-ionizing radiation [5].

1.2 Ionizing radiation

Ionizing radiation (IR), as the name indicates carry sufficient energy to remove electrons from atoms or molecules. It can be in particulate or electromagnetic form. The particulate forms consist electrons, protons, neutrons, α -particles etc. and the electromagnetic form includes as cosmic rays, X-rays, gamma rays etc. [5]. Ionizing radiation exposure may cause tissue injuries to the biological system

via biochemical, cellular and molecular targets leading to cellular and molecular damages such as oxidative damage to DNA, lipids and proteins as shown in **Figure 1** which may further lead to systemic damage [6, 8].

1.3 Types of ionizing radiation

1.3.1 Alpha particles

An alpha ray consists of two protons and two neutrons. These rays have a strong nuclear force and have the ability to bind to the nucleus of any atom. Due to their charge and mass, alpha particles interact strongly with matter and only travel a few centimeters in air. Alpha particles are unable to penetrate the outer layer of dead skin cells but are capable of causing serious cell damage if an alpha emitting substance is ingested in food or air [5].

1.3.2 Beta particles

Beta particles are high-speed electron or positron emitted from the radioactive decay of an atomic nucleus such as potassium-40 during beta decay. These particles

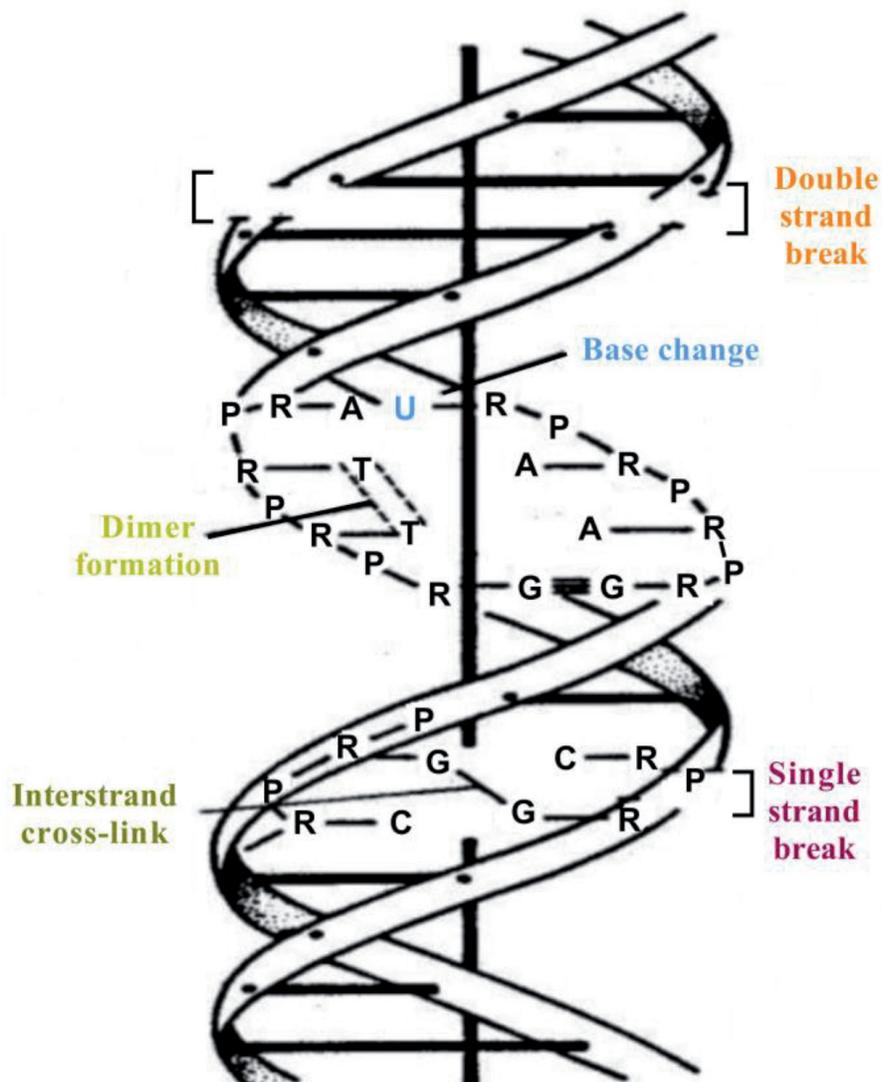


Figure 1.
Various type of DNA damage induce by radiation.

are emitted by unstable nuclei rich in neutrons, they are high energy electrons. These particles are negatively charged and have intermediate penetration power [5].

1.3.3 Gamma rays

Gamma radiation, unlike alpha or beta, does not consist of any particles; instead, they consist of a photon of energy being emitted from an unstable nucleus. These are produced by a change in the energy levels of the atomic nuclei. The wavelength of this radiation varies from 0.0003 nm to 0.1 nm. Gamma rays do not have any mass or charge. It can travel at much higher speed in air than alpha or beta rays and loses only half of its energy for every 500 feet. Gamma rays can be stopped by dense and thick layer of material such as lead or depleted uranium. These materials are used as an effective shielding in radiation related work [5].

1.3.4 X-rays

X-rays are generated from electron cloud when electron moves from higher energy level to lower energy level causing excess energy to be released. It is very similar to gamma radiation [5].

2. Effects of ionizing radiation on bio-molecules

Exposure to any types of ionizing radiations, whether man-made or natural have deleterious biological effects at any dose. Primary ionization of an atom in the biological system can induce either direct or free radicals mediated indirect damage. Radiation can damage the bio-molecules by both directly and or indirectly by generating free radicals (**Table 1**) [5, 14].

Among the bio-molecules damages, DNA damage has been shown to be most important and to contribute maximally to cell death [15, 16]. Studies made on DNA irradiated *in vitro* in solution, in the dry state or *in vivo* in the biological system have revealed that radiation causes a spectrum of damages to DNA. Among them, the important ones are an alteration of purine and pyrimidine bases, single and double strand breaks, removal of bases and crosslinking of DNA with DNA or adjacent protein molecules. When a cell is exposed to radiation reactive oxygen species (ROS) is generated which targets cellular DNA for base modification, DNA adducts, DNA single strand break and double strand breaks. All these alterations

Biomolecule	Damage
DNA	Loss of nucleotide and base modification, deletion of hydrogen bonds, sugar-phosphate bonds, DNA-protein cross linking, single or double strand break, guanyl, thymidyl and sugar radicals
Proteins	Degradation and modification of amino acids, cross linkage, denaturation, molecular weight modifications and change in solubility.
Lipids	Peroxidation and carbon bond rearrangement, conjugate diene and aldehyde formation, lipid cross-linking, increased microviscosity, cell membrane rupture.
Carbohydrates	Breakage of glycosidic Bonds and monomers, alcohol oxidation to aldehydes.
Amino acids	Generation of ammonia, CO ₂ , H ₂ S, Hydrogen molecules, Pyruvic Acid
Thiols	Redox reactions, radical formations, cross linkage.

Table 1.
Biomolecules damage by radiation exposure [9–13].

cause mutation and cell death (**Figure 1**). Endogenous genomic DNA damages are a relatively common event in the cellular life and if not repaired efficiently may lead to mutation, cancer, and cell death.

Radiation induced DNA damage can be divided into four categories [9, 10]:

1. Base damage
2. Alteration of sugar moiety
3. Cross-links formation of dimers
4. Single-strand breaks
5. Double-strand breaks

2.1 Base modification and damages

The most frequent modification is formation of hydroperoxide in the presence of oxygen. The most important one is hydroperoxidation of thymine [5].

2.2 Sugar modifications

Alteration in deoxyribose sugar is not very well understood and the alteration is (0.2–0.3 alterations of sugar per 10 SSBs. For this modification sugar is first oxidized and then hydrolysed followed by liberation of base, with or without breakage of phosphodiester bonds [5].

2.3 Cross-links and formation of dimers

Intra-strand crosslinks - between two parts of a single strand.

Inter-strand crosslinks - between the two strands.

Dimer formation - it occurs when two adjacent bases of single strands are joined by covalent bonds. It leads to the formation cyclobutane ring between them. Replication halts at the place where dimers are formed. Thymine-thymine dimers are most resistant and stable ones. They induce cutaneous cancers in the regions exposed to UV light [5].

There are approx 50,000 damage per day occurs inside the body due to the normal metabolic process such as maintenance of and replication of the genetic material. However, damage to DNA is native to life because its integrity is under constant attack from numerous endogenous agents such as free radicals generated during essential metabolic processes and from exogenous sources including radiation and chemicals. Endogenous damage affects the primary, rather than the secondary structure of the double helix. Four general classes of endogenous modifications can be envisaged as follows [11].

2.3.1 Oxidation

The oxidized bases formed as a byproduct due to oxygen metabolism show miscoding eg 8-oxo-7,8-dihydroguanine (8-oxoG), thymine glycol and similar oxidized bases [17]. Among these 8-oxoG is the most abundant and most dangerous one. It mispairs with adenine [18]. Strand interruptions are also generated by reactive oxygen species [19]. The spontaneous mutation rate due to single strand break is still unknown. Activation of poly ADP ribose polymerase (PARP) exerts most accurate response to single strand breaks [20].

2.3.2 Methylation

Some small molecules such as S- adenosylmethionine can methylate bases endogenously. According to recent study from almost 4000 residues generated per day 7-methylguanine (7 meG) is most important. 7-methylguanine base is relatively harmless and does not show any cytotoxic properties. Whereas endogenously produced 3-methyladenine (3-meA) which are few hundred in number are building block of DNA replication and should be efficiently repaired [21].

2.3.3 Hydrolysis

The base sugar bonds in DNA are relatively labile and several thousands of bases are lost each day in human cells under physiological conditions [12]. Purines are lost more easily than pyrimidines. Base loss sites probably represent the most frequent damage in human cells.

2.3.4 Mismatches

Mismatches can occur in DNA due to the incorrect incorporation by DNA polymerases, damage to the nucleotide precursors in the cellular nucleotide pool or by damage to DNA [13].

3. Single strand breaks (SSBs)

SSBs arise when diester bond between phosphate and the deoxyribose breaks. After the breakage of phosphodiester bond separation of both the strands occurs causing the water molecule to penetrate the breach. This process causes breakage of hydrogen bonds between the bases [5].

4. Double strand breaks (DSBs)

When two complementary strand of double DNA breaks in a location at a point less than 3 nucleotides is known as DNA DSBs. DSBs are considered as the most deleterious type of damage because both the complementary strands are damaged and it is very difficult for the internal repair mechanism of the cell to handle this type of damage. The factors leading to the formation of DSB include endogenous factors that are associated with physiological processes occurring in the cell and the exogenous ones [22–24].

In the presence of endogenous DNA damage, a cell can survive up to some extent, however the concentrated damages accelerated by exogenous agents such as ionizing radiations, radiomimetic drugs, ultra-violet radiations, and carcinogens can induce permanent changes. These changes lead to cancer or severely impaired cellular functioning and poor repair efficiency which may eventually cause cell death by triggering apoptosis or irreversible cell growth arrest [25]. Ionizing radiations generate ROS, which cause oxidative damage to DNA. The most important ROS are $O_2^{\cdot-}$ (superoxide radical), OH^{\cdot} (hydroxyl radical) and H_2O_2 (hydrogen peroxide). The highly reactive hydroxyl radical (OH^{\cdot}) reacts with DNA and as a result, various forms of DNA damage occur. Exposure of DNA to ionizing radiations result in a number of different lesions in DNA such as base damage, single strand breaks and double strand breaks [9, 10, 26]. DNA DSBs present a major threat to the integrity of chromosomes and viability of cells. Unrepaired or incorrectly repaired DSBs

may lead to translocations or loss of chromosomes, which could result in cell death or uncontrolled cell growth. In addition, adjacent single-strand breaks in opposite strands may be converted to double strand breaks upon replication. DSBs are lethal unless repaired [27]. Ionizing radiations also induce clustered DNA damage in cells, which symbolize two, or more lesions formed within one or two helical turns of DNA and are in part responsible for the biological effects of ionizing radiation. The damage includes DSBs and non-DSB clustered damage such as SSB formed in close proximity to additional breaks or base lesions on both strands. An increase in the ionizing density of radiation increases the complexity of clustered DNA damage leading to decreased reparability of DSB in cells [28].

5. DNA DSB repair

Humans cells have two major DSBs repair mechanisms i.e. homology directed repair (HDR) and non-homologous end joining (NHEJ) [23]. However, in recent years a new mechanism called as alternative non-homologous end joining (A-NHEJ) has evolved (**Figure 2**). The selection criteria for DNA repair mechanism depends upon cell type, cell cycle phase and damage threshold. The non-dividing cells do not have the option of undergoing HDR but dividing cells can use all the three repair mechanisms with some conditions. The condition is NHEJ and A-NHEJ both can act in all the phases of cell cycle, however, the HDR is only able to act at S/ G2 phase of the cell cycle [29].

5.1 Homologous recombination pathway

Homologous recombination pathway (HR) generally repairs the DNA lesions in late S or G2 phase of cell cycle. HR pathway is a series of interrelated pathways that participate in the repair of different types of DNA damages like double strands breaks (DSBs), interstrand cross links and DNA gaps. Several studies have shown

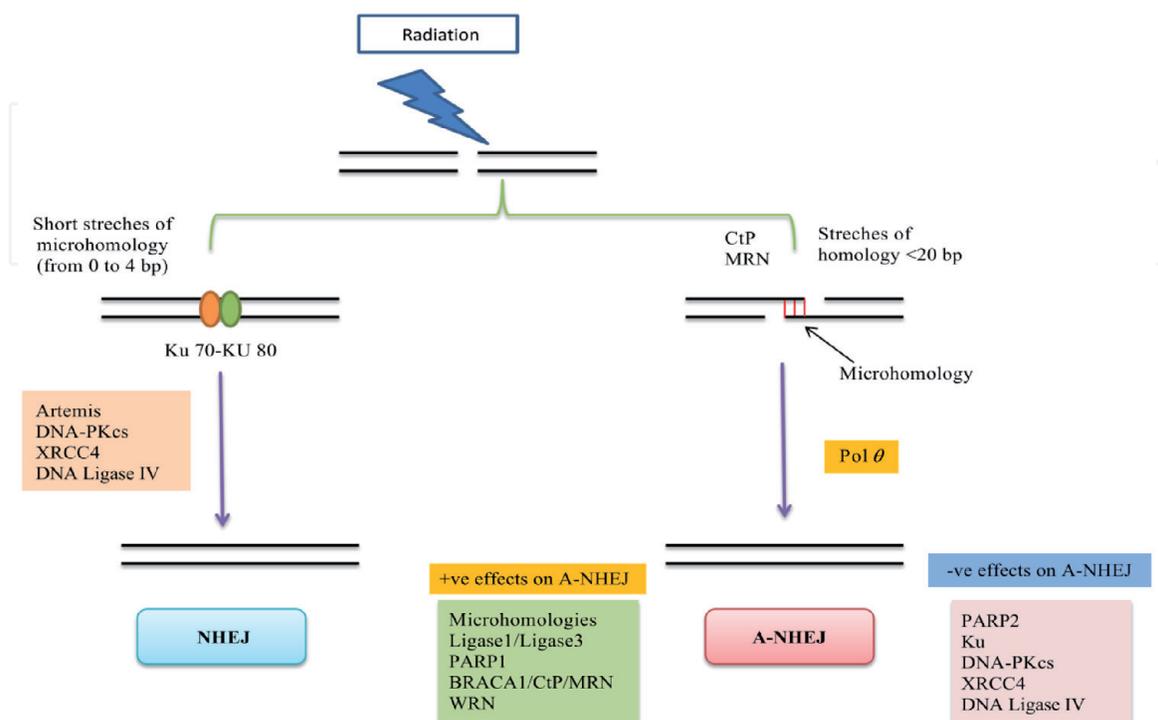


Figure 2.
 Double strand break repair pathway choice.

that HR is an error-free pathway. This pathway is known as error-free because it occurs only S and G2 phases of cell cycles. In these phases of cell cycles sister chromatids are more easily available and can be used as template to synthesize new strands of DNA [30]. HR pathway is essential for cell division in higher eukaryotes to prevent recombination between non identical sequences. HR plays an important role in DNA replication for duplicating the genome and also in telomere maintenance for the recovery of broken replication fork [31–34].

HR accomplishes through following steps:

1. At the end of DSBs processing nucleolytic resection occurs to generate 3' single-strand overhangs with 3-OH ends. This entire process makes use of MRN complex which has 3' to 5' exonuclease activity. 3' single-strand overhangs are generated by this exonuclease activity [35–37].
2. Formation of a recombinase filament on the ssDNA ends: The broken DNA ends has 3' single-stranded region which is coated with single strand binding protein, RPA. This binding of RPA removes secondary structures. After this, BRCA2 replaced RPA with the help of Rad51. Rad51 protein can interact with many ssDNA binding proteins like BRCA2, RPA, PALB2 and RAD52. Rad51 is 339 amino acid proteins that play an important role in homologous recombination of DNA during DSBs. Rad51 protein forms a helical nucleoprotein filament around DNA. The basis for Rad51 nucleoprotein filament formation to explore the homologous sequences on the sister chromatid [38–40].
3. A displacement loop (D-loop) intermediate is formed by strand invasion into homologous sequence. This invasion is prompted by Rad51, which enhances the activity of another protein Rad54B that facilitates D-loop formation by Rad51 in turn. However in meiosis the recipient DNA is similar but not identical homologous chromosome. D-loop is formed between homologous chromosome and invading 3' overhang strand [38, 41].
4. Formation of holliday junction: The holliday junction is a biological process that can increase genetic diversity by homologous recombination, shifting gene between homologous and nonhomologous chromosome as well as site specific recombination. This process also involved in DNA DSBs repair pathways. D-loop structure is further changed into cross-shaped structure, known as holliday junction. This occurs after adding of new nitrogenous base to 3' end of invading strand by DNA polymerase enzyme. This process ultimate leads to restoration of DNA strands on homologous chromosome. The junction is resolved after the restoration of lost sequences information and give error free repaired DNA. The double holiday junction model explained the resolution steps can be carried out by formation of two holliday junction to provide cross-over and non-crossover products [42–44].

5.2 NHEJ (non-homologous end joining) repair pathway

The classical NHEJ is a pathway that repairs DSBs. This pathway is generally active in all stages of cell cycles. In NHEJ, the breaks ends are ligated without the need of homologous template. This pathway is very prominent in G0 and G1 phases of cell cycles to repair up to 85% DSBs formed by IR. These breaks formed by IR are very complex and contain non ligatable end groups [45–48].

NHEJ pathway carried out in following steps.

5.2.1 Detection of the DSBs and tethering of the DNA ends

The first step of NHEJ is detection of DSBs site by Ku70/80 proteins. Ku70 (69.8 kDa) & Ku80 (82.7 kDa) is an important heterodimeric complex involved in NHEJ pathway [49]. This dimer is a central DNA binding core and helps in binding of broken ends of DNA with higher affinity. This binding leads to the formation of a bridge between two proximal DNA ends which may help in tethering of the broken ends of damaged DNA [50]. This heterodimer has toroid shape with large central ring to accommodate duplex DNA ends [51, 52]. The inner portion of the central ring is lined with positively charged amino acids. These positively charged amino acids interact with phosphodiester backbone of DNA ends in order to safeguard it from nucleolytic degradation. Ku70 and Ku80 contain unique amino (N) and carboxy (C) terminal regions. The N terminus is phosphorylated by DNA-PKcs and last 12 amino acids of carboxy terminal region of Ku80 is required for interaction of DNA-PKcs with Ku heterodimer [53]. The Ku70 proteins is mandatory for chromosomal organization. The carboxy terminal region of Ku70 is involved in chromosomal organization. The carboxy terminus of Ku70 proteins contains SAP domain (SAF-A/B, Acinus, and PIAS) [54, 55]. The binding of Ku protein with DNA leads to the conformational change in the C terminal region of Ku70 and Ku80. This conformational change facilitates interaction of Ku proteins to other proteins such as XLF, DNA-PKcs, Ligase IV complex, XRCC4 and DNA polymerase μ etc. [55–60]. Thus Ku proteins considered as the corner stone of this pathway. The first protein to interact with Ku is DNA-PKcs. It is also involved in tethering of DNA ends at DSBs which further facilitate the recruitment of other repair proteins [61]. The molecular weight of DNA-PKcs is 469 kDa and contains 4128 amino acids and it is largest protein kinase which is specifically activated by binding to duplex DNA [62]. The conserved region in the extreme C -terminus of Ku80 mediates interaction with C-terminus region of DNA-PKcs. The interaction between DNA-PKcs.Ku further allows DNA-PKcs to interact across the DSB by the formation of (DNA-PKcs- Ku-DSB complex or DNA-PK) Synaptic complex which serves to tether the broken ends of the DNA [50].

DNA-PKcs has weak serine threonine kinase activity and it is enhanced by DSB ends and Ku proteins. DNA-PKcs has weak serine threonine kinase activity and it is enhanced by DSB ends and Ku proteins. DNA-PKcs are when autophosphorylated leads to the liberation of DNA ends for processing and ligation. There are sixteen site that has been reported as autophosphorylation sites in DNA PKcs [63, 64]. Auto-phosphorylation of threonine 2609 and serine 2056 cluster play major roles in NHEJ process. It has been reported that radiosensitivity increases when phosphorylation of entire serine 2056 is inhibited whereas DNA ends processing is accelerated when there is phosphorylation at threonine 2609 [60, 63, 65–67]. The endonucleolytic activity of Artemis and ligation function of Ligase IV also supported by DNA PKcs [68].

5.2.2 Processing of DNA ends to remove damaged/non-ligatable groups

The next step after the detection of DNA ends in NHEJ is processing of the DNA termini to remove non ligatable end groups along with other lesions. Breaks in the DNA induces by IR are complex and depending on the nature of breaks require different processing enzymes like Artemis, DNA polymerase μ/λ , PNK etc. [69].

Artemis has 5'-3' exonuclease activity however upon complex formation with DNA -PK, it acquires endonuclease activity as well. This acquisition of endonuclease activity helps in opening DNA hair pins during V(D)J recombination [70, 71]. In the processing in DNA the gaps induce by IR are filled by DNA polymerase. The enzyme that plays pivotal role in NHEJ are DNA polymerase μ and λ . These are recruited at DSBs sites by complexation with Ku proteins. Both polymerases

are recruited to the DSBs site only when they interact with Ku proteins. They both carry out reactions for gap filling and the only difference in them is requirement of template DNA. Polymerase λ is template dependent whereas polymerase μ is not so much dependent on template DNA. After gap filling proteins such as APLF, PNK, WRN etc. remove non-ligatable ends. The APLF removes non-ligatable ends by exonuclease and endonuclease activities. Whereas PNK removes non-ligatable DNA ends by its 3'-DNA phosphatase and 5'-DNA kinase activities. WRN is a member of RecQ helicase family and removes non-ligatable DNA ends by DNA dependent ATPase, 3'-5' DNA helicase and 3'-5' exonuclease activities [72].

5.2.3 Rejoining of the broken ends of DNA

For the completion of DNA repair process, the broken ends of the processed DNA must be rejoined. In NHEJ pathway, the rejoining and ligation step is carried out by Ligase IV, an ATP dependent enzyme. Ligase IV forms phosphodiester bonds between broken ends of DNA and catalyzing the ligation step. After hydrolysis of ATP, covalent linkage of AMP moiety occurs at specific lysine residue in the active site of DNA ligase. After linkage there is release of pyrophosphate [73]. This process releases AMP. Ligase IV has two C-terminal BRCT domains and is separated by a linker region. The linker region of Ligase IV interacts with the alpha helical region of XRCC4 to form an extremely stable complex. Till date, there are no published data on enzymatic activity of XRCC4 in DNA repairing process. XRCC4 is an important mediator for the recruitment of various NHEJ factors to the site of DNA damage and accelerate the process of DNA repair. Previous studies on NHEJ pathway in DNA repair process support the role of XRCC4 in stabilization and enhancement of DNA Ligase IV enzymatic activity [73–75]. DNA Ligase IV rejoins one strand of DNA at a time and simultaneously recruits and activates other repairing proteins that responsible for ligation of opposite strand of DNA.

6. Alternative NHEJ pathways

Recent studies identified an alternative repair pathway for DNA DSBs and also known as alternative NHEJ (A-NHEJ). The drawback of alternative pathway is that, it is very slow as compare to C-NHEJ [29]. This pathway is only activate when all other repairing pathways fails to repair DSBs. Because of this, A-NHEJ pathway is also considered as backup pathway for NHEJ (B-NHEJ). In A-NHEJ, the broken ends of DNA are ligated by Ligase III and Ligase I [76, 77]. In 2011, Odell ID et al. explored the effectiveness of Ligase III in repairing DSBs. Ligase III is more effective than Ligase I because Ligase III interact with ERCC1 and PARP1. XRCC1 promote efficient base excision repair and PARP involved in base excision and single strands breaks repair [78]. Apart from this, XRCC1 and PARP1 can also be used as bio-markers to sense the repairing process by A-NHEJ pathway [75]. A recent study illustrates the compromising of A-NHEJ pathway by existing by C-NHEJ factors like Ku proteins etc. A-NHEJ is basically a backup pathway which is activated when NHEJ pathway is compromised. NHEJ pathway is compromised due to absence of one or more core component such as DNA Ligase IV, Ku70/Ku80 heterodimer. A-NHEJ requires single stranded DNA at the ends so certain recombination proteins such as MRE 11A and CtIP act in this pathway [79]. Mutation in NHEJ pathway is extremely rare which makes it difficult to understand whether a-NHEJ is standing pathway or the components involved in this pathway also have its utility in replication, recombination or repair. a-NHEJ require pol θ along with poly (ADP-ribose) polymerase I(PARP), MRN complex and CtIP [79]. A-NHEJ starts when

phosphorylated CtIP stimulates MRN complex for its endonuclease activity which generates 15-100 nucleotide 3' overhangs. NHEJ requires short microhomology of 0–4 bp whereas A-NHEJ requires microhomology of <20 bp. The annealing of the two 3' overhangs is stabilized by pol θ that is sealed by DNA ligase I or DNA ligase III. Apart from these functions pol θ also has transferase activity to add nucleotide to provide microhomology that is absent. Insertion of short templates are not necessarily involved with microhomology but also in human lymphoid translocation around (20–50%) [80]. There have been certain evidences that show pol θ activity when long 3' ssDNA tails generated by the process of extensive resection embeds annealed microhomologies. This process generates non-homologous 3' ssDNA tail that is needed to be removed before extension by pol θ [81]. So during A-NHEJ pathway there may be requirement of nuclease activity from other pathways as well as seen in mammalian system in xeroderma pigmentosum group F (XPF). XPF uses ERCC1 nuclease complex, APLF or Artemis-DNA-PKcs. Therefore it may be noted that proteins required for A-NHEJ are PARP1, the MRN complex and its partner CtIP and for end joining either LIG1 or LIG3 [25].

There is a possibility that A-NHEJ is slower than NHEJ as seen in class immunoglobulin class switch recombination where missing DNA ligase IV can be replaced with DNA ligase I or DNA ligase III. This substitution occurs but with tenfold slower kinetics [82, 83]. This substitution of DNA ligase IV with DNA ligase I or DNA ligase III suggests presence of backup components of such important enzymes but of lower repair efficiency and with slower kinetics. Future work is needed to identify all the differences between NHEJ and A-NHEJ and also the component of A-NHEJ. Not only distinction but also the repair kinetics is also an important point to be taken into consideration. The fine balance between NHEJ and A-NHEJ is also mediated by ataxia telangiectasia mutated - mediated DNA damage response. In the absence of ATM NHEJ is favored. There is extremely rare and lethal for mammals that lack components for NHEJ [84]. Therefore it should be noted that components for A-NHEJ i.e. its enzymes and proteins may have other functions as well apart from being a substitute. So according to Dueva and Iliakis 2013 there are two models through which A-NHEJ is activated. The first one states that A-NHEJ comes into play when NHEJ or HRR which were engaged for the repair of double strand break but failed to complete the process. According to the second model states that A-NHEJ comes into action when either of the process NHEJ or HRR attempted for the repair mechanism but somehow failed [29]. Basically A-NHEJ comes into play as a backup process for NHEJ or HRR with slight differences. When A-NHEJ backs up the failure of NHEJ it can occur throughout the cell cycle as NHEJ is active throughout the cell cycle. But when it backs up the shortcomings of HRR it can only occur in S- and G2- phase of the cell cycle. This type of repair pathway contributes to 10–20% of radiation induced DSBs [34, 85]. A-NHEJ basically operates on resected end that inactivates NHEJ and paves way for HRR which truly justifies the dependences of A-NHEJ on certain proteins such as MRN complex, CtIP, BRCA1 [86, 87].

6.1 Role of A-NHEJ in leukemia progression

Leukemia and lymphoma are the type of cancer that shows translocation of chromosomes with involvement of A-NHEJ [88, 89]. There is an availability of evidences that show A-NHEJ play active role in erroneous repair of programmed DSBs during V(D)J AND Class Switch Recombination (CSR) [29]. Severe combined immunodeficiency syndrome (SCID) is a disease which occurs due to mutation in DNA repair proteins [90]. SCID like phenotype is observed in murine models that lack RAG proteins [91–93]. These murine models are also seen to develop tumors because of the translocation in Ig locus due to A-NHEJ. A model was also proposed

which suggests A-NHEJ mediated genomic instability was suppressed with the help of RAG1/2 proteins and NHEJ factors [94, 95]. RAG complex formed post cleavage shunts the broken ends of DNA to NHEJ thus suppressing recombination events. It is seen that RAG mediated DSB repair during CSR is not compromised in cells lacking NHEJ but is shifted to A-NHEJ [82, 96, 97]. There is effect of absence of DNA-PKcs and it uses Lig1 or Lig3. XRCC1 which acts together with Lig3 is not necessary for A-NHEJ during CSR. In fact the absence of these components increases CSR efficiency [98, 99]. PARP1 and PARP2 is nonessential component during CSR but PARP1 favors A-NHEJ whereas PARP2 suppress translocation during CSR [100]. It is very interesting to note that in chronic myelogenous leukemia (CML) there is increased production of ROS due to increased cell division which is facilitated by BCR-ABL tyrosine kinase. Increased ROS inside the cells leads to DNA damages especially DSB. This leads to the up-regulation of A-NHEJ [101–103]. The cells which are BCR-ABL positive CML shows up regulation of key proteins for A-NHEJ i.e. Lig3 α and WRN whereas down regulation of key proteins of NHEJ Artemis and Lig4. Therefore A-NHEJ enables the cells of CML to repair ROS induced DSB and survive. Though this repair pathway of A-NHEJ is error prone the price the cells pay for survival is genomic instability [104].

In acute myeloid leukemia (AML) mutation that occurs are internal tandem duplication (ITD) of FMS-like tyrosine kinase3 (FLT3) receptor. FLT3-ITD is type of cancer which utilizes microhomology mediated A-NHEJ to repair double strand breaks. It causes increased number of deletion. The cells expressing FLT3-ITD has increased protein level of Lig3 α but decreased level of Ku protein required for NHEJ. This causes shift towards the A-NHEJ for DSB repair [105].

6.2 Targets for cancer therapy

PARP1 inhibitors could act as therapeutics for cancer in BRCAness (**Table 2**). Certain therapeutic strategy involves the use of DNA ligase as targets [106]. In BCR-ABL-positive CML it is treated with tyrosine kinase inhibitor Imatinib, this strategy immense hope for targeting A-NHEJ factors for therapeutics. Tobin et al. reported that BCR-ABL-positive CML resistant to Imatinib were sensitive to combinational treatment of Ligase and PARP inhibitors which correlates with hyperactive A-NHEJ [109]. This therapy was effective in therapy resistant breast cancer cell lines as it became

Cancer	Genetic Background	Drug targets	Altered Repair pathway	References
MCF7 breast cancer	Reduced DNA LIG4, Enhanced DNA LIG3a and PARP1	PARP1 with DNA ligase inhibitors	NHEJ	[25, 106, 107]
Chronic Myeloid leukemia	BCR-ABL, enhanced expression of LIG3a, PARP1, and WRN	PARP1 with DNA ligase inhibitors	HR	[72, 102, 108]
Breast, Ovarian	BRCA 1 deficient BRCA 2 deficient	PARP 1 PARP 1	HR	[38, 106]
Non-BRCA1/2 breast cancer	XRCC4 deficient	unknown	NHEJ	[56–59, 73, 74]
Leukemia, proB-cell lymphoma	KU, P53 deficient	Unknown	NHEJ	[49, 53, 58, 79, 105]

Table 2.
Disease, impaired repair pathway along with their therapeutic targets.

sensitive to DNA ligase and PARP inhibitors [107]. Many PARP inhibitors obstruct DNA replication by trapping PARP [108]. Lig3 α or PARP inhibitors are also included in novel therapeutic strategies for AML associated with FLT3 mutations [105].

Therefore there is an immense possibility of treatment of cancer with A-NHEJ inhibitors which involves tumors with increased A-NHEJ. And it will be interesting to see if there is possibility of protecting an organism from carcinogenesis by limiting the function of A-NHEJ.

7. Conclusion

Radiation and other assaults that cause DNA damage leading to double strand break are dealt by the mammalian system by relying on tightly regulated repair pathways that are end-joining or recombination-based repair pathways. These are highly regulated repair pathways and results in accurate restoration of the genome. Error prone double strand break repair is still prevalent despite of its mutagenic potential. We must also understand that it is not simply a backup mechanism that comes into play when accurate repair pathway is not possible. The various factors that regulate it are cell cycle stage, local sequence context (homology), and genome structure. So the error prone repair pathway is also very important as it prevents major genome catastrophe. Detailed survey of literature puts forward the fact that error prone pathway paves way for genome evolution in somatic tissues in context of cancer. It is apparent that clear understanding of how A-NHEJ operates and is regulated inside the cell after double strand break will have important therapeutic implication in context of cancer treatment and cure.

Acknowledgements

We would like to thank all researchers for their contribution in the field of radiation and sharing the final outcome of their hard work in this field with entire world.

Conflicts of interest

The authors declare there are no conflicts of interest.

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