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DNA Repair Pathways are as Novel Therapeutic Targets

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Abstract

Oxygen is essential for life however it may be toxic and mutagenic through the production of reactive oxygen species (ROS) including free radicals. ROS generation can be considered a double-edged sword. It is known as the oxygen paradox (good or bad effects of oxygen). ROS can give damage to biomolecules such as DNA, RNA, protein and lipids. Most of damages can repair by DNA repair systems and antioxidant defense system which a have major roles to eliminate ROS. DNA repair systems also have major roles in the development of many diseases, notably, cancer. Cancer is the second most common cause of death in the US, exceeded only by heart disease. Cancer accounts for nearly 1 out of every 4 deaths in the United States. This year, about 600,920 Americans are expected to die of cancer – that's more than 1,650 people a day. The financial costs of cancer are high for both the person with cancer and for society as a whole. The Agency for Healthcare research and Quality (AHRQ) estimates that the direct medical costs (total of all health care costs) for cancer in the US in 2014 were $87.7 billion. The increasing concern about healthcare costs, use of DNA repair inhibitors in cancer therapy can be effective and providing its targeted therapy close to us personalized medicine. Poly (ADP-ribose) polymerase Inhibitors (PARPi), one of the DNA repair enzymes, is the first drug used in cancer therapy. In the present review, the process from DNA repair to use as anti-cancer drug is explained in a clear and understandable manner.

Keywords

DNA damage, DNA repair, PARP inhibitors, Cancer.

1. Introduction

Oxygen is the key for generating energy in cellular respiration. Furthermore, oxygen can give damage to DNA through the production of Reactive Oxygen Species (ROS) including free radicals. The overproduction of ROS and the insufficiency of an antioxidant mechanism results in oxidative stress (OS) **(1).** Increased OS is a widely associated in the development and progression of diseases and their complications. They are usually accompanied by increased production of free radicals or failure of antioxidant defense **(2).** DNA damage is chemical modifications in DNA structure by normal metabolic activities and environmental factors. Reactive Oxygen Species (ROS) including free radicals can occur as by-products of mitochondrial respiration. Briefly, in our body, oxygen is reduced to water during normal metabolic activities/process. This conversion occurs 98% from oxygen to water however 2% leakage occurs thus it is formed Reactive Oxygen Species (ROS) including free radical. They attack to DNA and cause damage to DNA and other biological molecules. (Endogenous damage). Environmental agents attack to DNA and other biological molecules and change their structure (Exogenous/Environmental damage).

Environmental sources of damage can be physical such as ultraviolet (UV) light, ionizing radiations (IRs) or chemical such as chemotherapeutic drugs, industrial chemicals, and cigarette smoke **(3).**

Given the size of the human genome and the large number of cells in a human body (about 3.7 × 1013) errors will inevitably accumulate during the lifetime of an individual. Most of these errors will remain silent, but they can also cause serious diseases. It takes place ~ 3 x 1017 damages / day **(4).** It takes place 60 000 in a day and 2500 in an hour DNA damaging events. Mutations, is a change in an organism's DNA sequence, are inevitable even with very powerful repair systems. The human haploid genome is 3.2 × 109 base pair. It means that on average there are 0.32 mutations introduced every time the genome is replicated **(5)**. Life has a delicate balance between genomic stability and the necessity of genetic instability (the formation and persistence of mutations). The balance in the context of genomic stability must be precisely regulated and controlled **(6).** To achieve this aim, a number of multiple and overlapping DNA repair pathways have been prepared within the cell therefore, life will continue. If the DNA is not repaired, the mutations occur thus leads to cancer and hereditary diseases. After these numbers on damages, it is realized that DNA repair is how important and essential for maintenance of genome integrity. After healthy cells are attacked by external or internal agents, there are 2 possibilities. DNA is repaired by repair systems or not repaired. Afterward, cells with damaged DNA again are attacked by these agents. This time, DNA damage

accumulates in cells and induces apoptosis and leads to diseases and cancers **(7).** The toxic and mutagenic results of such damages are minimized by distinct DNA repair systems namely base excision repair (BER) and nucleotide excision repair (NER) so on. DNA repair systems generally involve the removal of damaged or incorrect bases, and require a DNA polymerase to resynthesize DNA. Because preserving the genome is paramount, DNA repair is replete with alternate plans. If one pathway fails to repair a problem, another pathway can start to work. There are many repair proteins which have major roles in these repair systems **(8)**.

After all these improvements on genome, researchers started to concentrate on studies related to DNA repair enzymes. In 1994, DNA repair enzymes are selected the "Molecule of the Year" because of their roles on DNA repair and having large individual differences. They become a cover of "Science" journal **(9).**

According to the PubMed database, when "DNA damage" and "DNA repair" keywords were used, there were a few published studies in 1972 and at the present time, the number of published studies reached about 2500.

When "DNA repair" and "DNA repair inhibitor" keywords are used, it is observed that while there has no work until 1996, 500 published studies at the present time. These numbers demonstrate the importance of DNA repair.

Cancer is a class of diseases characterized by abnormal cells that grow and invade healthy cells. Mutations and changes in the genetic code cause cancer. Mutations in genes cause changes in proteins. Most mutations occur in somatic or germline cells. Cancer cells differ from normal cells in many ways. While in normal tissues, the rates of new cell growth and old cell death are kept in balance, cancer tissues, this balance is disrupted. This disruption causes uncontrolled cell growth or apoptosis. DNA damage-repair cycle in cancer cell differs from normal cell. Tumour cells, because of the frequency of their replication and their genomic susceptibility, have increased frequency of mutations. Replication is replaced by proliferation in cancer cells and trigger uncontrolled growth **(10).** An elevated activity of repair pathways can significantly decrease cancer cells' sensitivity to many known anticancer agents and, consequently, increase their antitumor drug resistance. The aim of cancer therapy is to prevent or stop cell proliferation or kill cancer cells to give damage to cells. Chemotherapy or radiotherapy kill cancer cells however, rapidly growing tumours accumulate

mutations that lead to overexpression of DNA repair genes, increasing DNA repair capacity. It is clearly shown that, the repair capacity of cancer cells is higher than normal cells.

One important mechanism by which cancer cells can develop resistance to therapy is to increase their DNA repair capacity by overexpression of DNA repair genes. DNA repair inhibitor are started to use cancer therapy. DNA repair mechanisms affect the response to cytotoxic treatments. Therefore, it is important to understand the mechanism of repair systems and interfering repair processes to induce tumour death which is the aim of DNA repair inhibition.

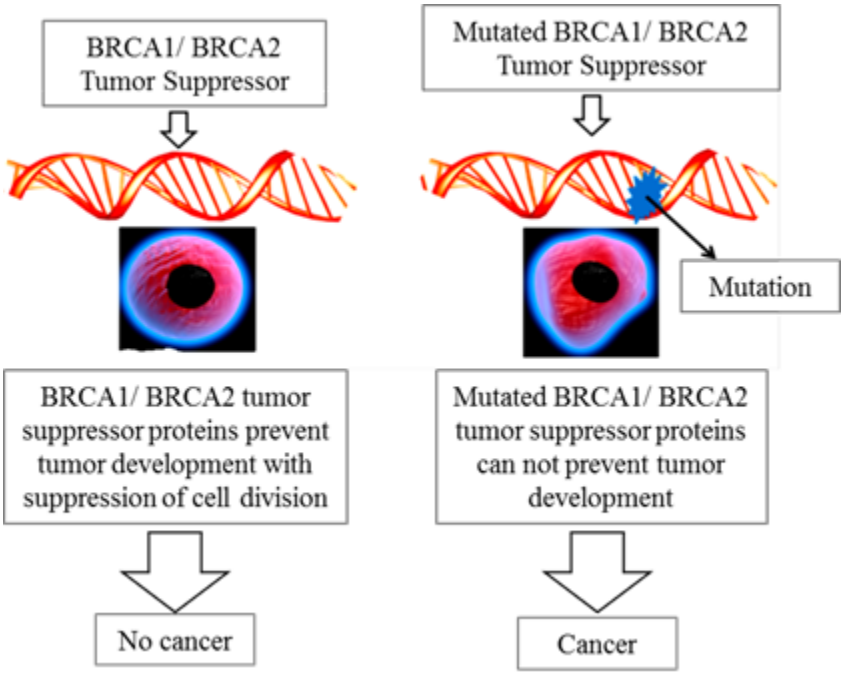
The increasing understanding of the functions of PARP enzymes in DNA repair has led to the exploration of specific inhibitors of PARP in the cancer therapeutics setting. Poly (ADP-ribose) polymerase (PARP) is key enzyme in normal cellular process of single strand DNA, which was first described over 50 years ago **(11,12)**. The lack of simultaneous loss of both genes causes cell death. In this status, synthetic lethality takes place between two genes to continue to life. In another word, when DNA damage occurs, it can be repaired by DNA repair systems. If one pathway fails to repair a problem, other system can start to work. If the alternative pathway contains a mutation that makes the pathway dysfunctional or non-functional, then impairment in repair pathway can force repairs into the backup mode in this point, causing the cells to self-destruct. That is based on synthetic lethality. Synthetic lethality has provided new opportunities for the development of targeted cancer therapies. It was the first example that PARP-BRCA interaction successfully used this approach in the cancer therapy. Now, it is used in clinic **(13)**.

Base Excision Repair (BER) is different process from Single Strand Breaks (SSB). PARP-1 protein repairs SSB and is one of the first proteins to bind the lesion. The PARP proteins compose of 17 enzymes, which have many functions such as DNA transcription, DNA damage response, genomic stability maintenance, cell cycle regulation, and cell death **(14)**. During BER process, SSB occur and PARP transiently involve to BER process by binding these SSB. When PARP-1 is inhibited, it can be trapped on the SSB intermediate and prevent the ligation step **(15)**. Inhibition of PARP activity thus leads to an accumulation of unrepaired SSBs that in proliferating cells result in stalling and collapse of replication forks and, consequently, to DSBs. A double-strand break (DSB) can be repaired by homologous recombination (HR) or non-homologous end joining (NHEJ). DSBs, if not repaired by HR, result in cell death due to mitotic catastrophe. BRCA 1 and BRCA 2 are tumour suppressor genes involved in the HR pathway DNA repair of DNA DSBs **(16)**. (Mutations in BRCA1/BRCA2 genes result in defective HR and increase risk of developing breast and ovarian cancers **(Figure 1)**.

Inhibition of PARP1 activity leads to an accumulation of SSB that are converted to DSB but cannot be repaired by HR, resulting in increasingly high levels of genetic instability and, eventually, cell death **(17)**

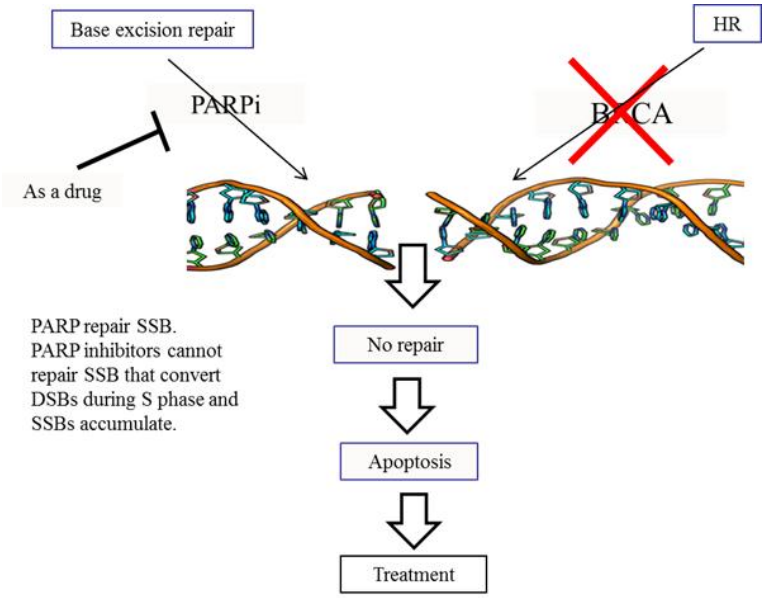
PARP inhibitors (PARPi) are a novel type of medication that works by preventing cancer cells from repairing their DNA once they have been damaged by other chemotherapy agents. PARPi is not only block SSB repair but also 'trapping' PARP at sites of DNA damage, resulting in a cytotoxic PARP-1–DNA complex. PARP inhibition and the PARP-1–DNA complexes are more cytotoxic than genetic depletion of PARP-1 **(18)**.

Figure (1): BRCA1/BRCA2 gene and cancer development



The first clinical study that demonstrated the benefit of the PARPi olaparib (lynparzaTM) as monotherapy in *BRCA*−/−patients was presented in 2007. The first cancer treatment targeted against an inherited genetic fault to be licensed. Inherited mutations in one copy of either the BRCA1 or BRCA2 gene is associated with a high risk of breast and ovarian cancer. Lost a functional copy of BRCA1 or BRCA2, are tumor suppressor proteins, causes to increase these cancers. These proteins are required for homologous recombination (HR) to suppress genetic instability. BRCA1 and BRCA2 defective tumors are intrinsically sensitive to PARP inhibitors **(19,20)**. **Figure 2** demonstrates the mechanism of treatment with PARPis.

Figure (2): BRCA pathway, the effect of PARPi to damaged cells and synthetic lethality



Damaged DNA can be repaired by two proteins: PARP and BRCA.

Mild side effects were observed by olaparib treatment. It selectively targeted BRCA defective cells owing to its defect in HR.

Olaparib (AZD-2281, trade name Lynparza) is an FDA-approved targeted therapy for cancer. It is a first-in-class, orally-active, poly (ADP-ribose) polymerase inhibitor. It induces synthetic lethality in homozygous BRCA-deficient cells **(21)**. Olaparib has a major efficacy to maintenance monotherapy in women with platinum-sensitive **(22)**. It stated that platinum-sensitive patients' recurrent serous ovarian cancer, maintenance monotherapy with the PARP inhibitor and therefore olaparib significantly improves progression-free survival versus placebo **(23)**.

Olaparib showed activity in advanced breast, ovarian and prostate cancers which were no longer responding to previous therapy in Phase I clinical trials. In 2009, phase II clinical trials of olaparib were initiated in breast, ovarian and colorectal cancer. Activity was seen in ovarian cancer, about 42% in patients with BRCA1 or BRCA2 mutations. In 2014, the FDA and the EMA approved Olaparib as monotherapy, at a recommended dose of 800 mg twice daily.

Studies on PARP is are drastically increased after olaparib has achieved a remarkable success in BRCA1 and BRCA2 breast cancer. The main advantage of olaparib has relatively mild adverse effects

compared with classic chemotherapy. It provides specific therapy through the targeted specific molecular defects or anti-cancer effect/action. This is proof of concept that most tumours of BRCA mutation carriers have antitumor response, in some cases even complete remission. This strategy increases the efficacy of treatment and reduces toxicities.

According to Clinical trials database, when search was performed for PARP inhibitor, there have been 223 studies on PARP inhibitors. Seventy six of 223 studies are conducted for ovarian cancer and 66 studies for breast cancer. In these clinical trials, monotherapy, several drug combination strategies with more than 50 candidate drugs have been tested such as, BMN673, CEP9722, ABT 888 (Veliparib), CEP-9722 is PARP-1 and -2 inhibitors. BMN, KU-0059436 (AZD2281) (PARP inhibitor), Veliparib, BKM120 and Olaprib Eighty-one studies are open and recruiting for clinical trials **(24)**.

2. Conclusion

The use of DNA repair proteins in cancer treatment has gained momentum with deciphering of human genome through Human Genome Project and accompanying that with developments of molecular analysis methods. It is aimed to manage patients better, increase efficacy-decrease adverse effects, and to assess patients' predisposition to diseases based on their genetic make-up provide the personalized treatment. The application of new treatment strategies should not only perform for cancer, but also for the treatment of other common and chronic diseases which have high healthcare costs. In future further strategies for cancer therapies need to be improved by targeting specific molecular defects in DNA repair systems that characterize certain cancer cells.

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