Combination Approach of Hyperthermia and Poly (ADP-ribose) Polymerase (PARP) Inhibitors in Cancer Treatment- A Brief Review

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Abstract

Cancer is among the leading causes of death worldwide. The key therapeutic modalities to treat cancer include chemotherapy, hormonal therapy, targeted therapy, immuno-therapy and combination therapy. A number of chemical agents target DNA; they cause breaks, intercalation and cross-links to damage DNA thereby inhibiting cell proliferation. Cisplatin and taxol based chemotherapies are still the most used chemotherapeutic agents which is commonly used for treating many different tumor types, including head and neck, lung, testis, ovarian, cervix and breast. In spite of being in the primary line of treatment modality, resistance to cisplatin is one the major limitation in cancer treatment. This is why the concept of combination therapies came into scene. Degradation of BRCA2 by hyperthermia causes abnormal localization of RAD51 which, in turn, attenuates the process of DNA repair via HR. Hyperthermia, in combination with PARP inhibitors is now a day is one of the best choices that is describes by several authors. This is an alternative combination to kill cancer cells causing minimum damage to normal cells.

This review explores why hyperthermia should be chosen as a better modality of treatment to treat cancer in combination with PARP inhibitors.

Key words: DNA damage repair, PARP, homologous recombination, hyperthermia, PARP inhibitors.

1. INTRODUCTION

1.1 DNA Damage

It is needless to emphasize the importance of maintaining the integrity of DNA. Yet, damages to DNA do occur. While certain damages can occur through exposure to different exogenous agents, certain changes can also occur spontaneously. DNA damages can also be induced either by physical agents like ultraviolet (UV) radiations from sun, exposure to x-rays or gamma rays [for medical diagnosis/therapy or nuclear fallouts], hydrolysis, thermal disruption or from exposure to chemical agents including certain plant toxins, hazardous environmental pollutants and chemicals (1).

DNA damage is considered to be the primary cause of cancer. DNA damage occurs from exposure to exogenous agents as well as from endogenous factors from altered/defective metabolic processes. The majority of the cancers are due

to environmental factors (90–95%); the remaining is due to inherited genetics (2).

1.2 DNA Repair

The damaged DNA is mainly formed due to single strand breaks (SSBs) and double strand breaks (DSBs). Eukaryotic cells possess different mechanisms to repair the damaged DNA, including nucleotide excision repair (NER), mismatch repair (MMR), base excision repair (BER) that repairs SSBs. The two key mechanisms to repair DSBs to restore the genomic integrity within the cells are non-homologus end joining (NHEJ) and homologous recombination (HR) error-free pathway (3, 4).

Among the different enzymes involved in DNA repair processes, poly (ADP-ribose) polymerase (PARP) is one of the most significant one as it has a role in several DNA repair pathways. Apart from its role in SSB repair via BER pathway, PARP is also involved in DSB repair with the help of NHEJ and HR pathway (4).

NHEJ is a Ku-protein dependent pathway which is inhibited by the hetero-dimers Ku 70 and Ku 80 that binds and activates DNA-PKcs that leads to the recruitment and activation of end-processing enzymes, polymerase and DNA ligase IV while HR repairs DSBs by using information from the homologous sequences as a blue print. HR is generally restricted to S and G2 phase because it uses the information from the sister-chromatid sequence. When DSB is converted into homologus recombination repair (HRR), the MRN complex initiates the repair process that includes other proteins such as CtIP, BRCA1, BLM and Exo1. After this, Replication Protein A (RPA) comes and binds the 3' overhang site which prevents the secondary structure formation and stabilizes the single-stranded DNA (ssDNA). RPA mediates the formation of Rad51 nucleoprotein filament, which forms the presynaptic complex with the aid of additional factors such as the tumor suppressor BRCA2, the recombination mediator Rad52 and the Rad51 paralogs (Rad51-B, Rad51-C, Rad51-D, XRCC2 and XRCC3) (18). Using a donor sequence as a template, the HRR DNA synthesis initiates and followed dissociation of RAD 51 from the complex (4).

1.3 Poly (ADP-ribose) polymerase (PARP)

The process of poly(ADP-ribosyl)ation is a post transcriptional modification on different proteins that is executed by the enzyme poly(ADP-ribose) polymerase. Apart from its role in DNA replication, PARP is a well-known DNA repair enzyme which has a role in different DNA repair processes. The PARP super family was discovered in early 1960's. The family involves 17 isoforms (5). Among these 17 members, PARP1, PARP2, PARP3, vault PARP (vPARP) and tankyrase are the important members of the family.

PARP1 is a 116KDa protein found in eukaryotes and is mostly studied. It is the most abundant nuclear protein that accounts for almost 85% of the cellular PARP activity. PARP1 protein is highly conserved. Human and mouse PARP1 has 92% homology at the amino acid level. The family use NAD+ as a substrate to transfer ADP-ribose onto glutamic acid residues of proteins. In addition to PARPs, the family contains distinct class of mono(ADPribosyl)transferase found in different bacterial toxins (6).

The primary structure of human PARP1 consists of 1014 residues which are organized into 3 functionally distinct domains:

(i) N-terminal DNA binding domain (DBD)- this domain contains two Cys-Cys-His-Cys-Zinc fingers (F1/Zn1 and FII/Zn2) that facilitates binding to DNA; a third FIII/Zn3 has been discovered that has a role in interdomain association for DNA-dependent enzyme activation and a caspase 3 cleavage site.

(ii) Auto modification domain (AMD)- this domain contains the BRCT motif which participates in various protein-protein interaction. Poly(ADP-ribosyl)ation of PARP occurs through this domain.

(iii) C-terminal catalytic domain (CD)- this Domain contains the enzyme's active site and this domain is highly conserved among eukaryotes. The active site is the PARP signature motif which binds to NAD^+ and also bears the "WGR" (Trp, Gly, Arg) motif (5).

1.4 Mechanism of action of Poly (ADP-ribose) polymerase (PARP)

PARP1 is triggered when cell senses DNA damage. PARP1 binds to both single and double strand breaks. After PARP1 binds to DNA through the second Zn-finger domain, PARP1 forms homodimer and catalyzes the cleavage of NAD^+ into nicotinamide and ADP-ribose and then uses these ADP-ribose units to synthesize polymers onto different nuclear acceptor proteins and also onto itself. The polymer may be linear or branched. The size of the polymer may range up to 200 ADP-ribose units. This high negatively charged branched polymer attracts the nucleosomal proteins away from the DNA to form a protein-polymerase complex which then dissociates from the DNA. This dissociation decreases the nucleosomal stability to facilitate the entry of enzymes that triggers DNA repair (7). There are two enzymes; poly (ADPribose) glycohydrolase (PARG) and ADP-ribosyl protein lyase that are involved in the catabolism of poly(ADP-ribose) (pADPr). PARG hydrolyses the linear or branched chain polymer when the polymer length stretches beyond 200 units. This helps the enzyme to resume its new cycle of auto-modification in response to DNA damage. ADP-ribosyl protein lyase removes the protein proximal ADP-ribose monomer. Poly(ADPribosyl)ation is a dynamic process where the polymer has very short in vivo life (<1 min)(7).

There are more than 30 nuclear proteins that act as an acceptor of pADPr units *in vivo* and *in vitro* (8). *In vivo*, the most abundantly poly(ADPribosyl)ated protein is PARP1 itself as it catalyses its own auto-modification to down regulate the enzyme's activity. Other than PARP1, histones are also considered to be the major acceptors of pADPr (7). The poly(ADP-ribosyl)ation confers negative charge to the histones, leading to electrostatic repulsion between DNA and histones (7). Other transcriptional factors are also involved that acts as an acceptor for pADPr. The list involves DNA replication factors and signaling molecules like: i)NF-κβ, ii) AP-2, iii) Oct 1, YY 1, iv) B-MYB, v) DNA dependent protein kinases, vi) p53, vii) topoisomerase I, viii) Lamin B, ix) B23 (9).

1.5 Role of PARP 1 in DNA repair

PARP1 becomes activated upon DNA damage. Among the various DNA insults, SSBs occurs more frequently at a rate of 104 per day. SSBs are repaired through combination of BER, NER and MMR pathways; predominantly by BER using PARP (10). PARP1 identifies the SSBs and then recruited at the site of damage as a homo-dimer. The PARP polymers form up to 10-500 folds within 30 seconds. PARP1 undergoes conformational changes in transferring the ADP-ribose moieties from cellular NAD^+ to the acceptor proteins which ultimately opens up the acceptor proteins from the DNA. The PAR chain is hydrolysed by PARG when more than 200 units of PAR have been formed (7). The negatively charged PAR chain along with the acceptor proteins dissociates from the site of insult allowing DNA repair enzymes to carry on the repair mechanism. The repair enzymes include DNA ligase III (Lig III), DNA polymerase β (pol β) and scaffold proteins like X-ray complementary gene1 (XRCC1) which forms the BER multi protein complex. Dissociation of BER complex makes the PARP1 inactive and halts the synthesis of PAR polymer (10). Shall and colleagues first observed the role of PARP1 in BER. They reported that when 3-amino benzamide (3AB) is used to treat cells, the ligation of dimethyl sulfate (DMS) induced single strand breaks occurs dramatically and there was increase in DMS cytotoxicity (11). Apart from its role in SSB repair via BER pathway, PARP1 is also involved in DSB repair with the help of NHEJ and HR pathway.

The key therapeutic modalities to treat cancer include chemotherapy, hormonal therapy, targeted therapy, immuno-therapy and combination therapy. A number of chemical agents target DNA; they cause breaks, intercalation and cross-links to damage DNA thereby inhibiting cell proliferation (12).Cisplatin and taxol based chemotherapies are still the most used chemotherapeutic agents which is commonly used for treating many different tumor types, including head and neck, lung, testis, ovarian, cervix and breast (13). The

basic mechanism of these drugs is based on formation of intra and inter strand crosslinks, SSBs and DSBs in replicating cells (13). The accumulation of unrepaired DNA lesions, particularly DSBs, can lead to cell death.

Several strategies have been implemented for efficient cancer therapies that depend on initiation of DNA damage to abolish malignant cells. One of such strategy is the inhibition of PARP1. PARP1 inhibitor targets the HR-deficient tumors. For better efficacy in cell killing, many results have suggested that hyperthermia can be used in combination with PARP inhibitors.

1.6 Hyperthermia

Hyperthermia is an anti-cancer treatment during which external heat sources are employed to treat tumors. During the treatment, specialized equipment is used to heat the tumor regionally to a final temperature in the range of 39-42.5°C which is considered to be non-lethal while temperature above 43°C is considered lethal. This is a safe and effective way to enhance the effectiveness of radiotherapy and some types of chemotherapy using cisplatin, carboplatin, cyclophosphamide, ifosfamide, melphalan and mitomycin C (14). Numerous studies have stated that hyperthermia can cause high percentage of damage to cancer cells with minimum effect in normal tissues. Different research groups worldwide are trying to explore local, regional, and whole-body hyperthermia (15).

1.7 Treatment of cancer with hyperthermia

There are mainly 3 mainstream modalities to treat cancer: (i) removal of tumors through surgery (ii) radiation therapy (iii) chemotherapy which has improved patient survival for different types of cancer, but still there is much more room for development of other better modalities. Over past few decades scientists and researchers have been struggling to bring some other strategies to fight cancer after considering that the mainstream modalities of treating cancer can cause several side effects. The other modalities include hyperthermia (thermo-chemotherapy), biological therapies like immunotherapy, photodynamic therapy, laser treatment, gene therapy, and inhibitors of angiogenesis (15).

1.8 Intracellular changes occurring due to hyperthermia

(a) Hyperthermia causes failure of cellular homeostasis (16). The main event is protein denaturation and aggregation, resulting in cell cycle arrest, inactivation of protein synthesis, and inhibition of DNA repair processes. Other cellular defects due to hyperthermia include: (1) the inhibition of DNA synthesis, transcription, translation; (2) degradation and misfolding of proteins through the proteosomal and lysosomal pathways; (3) cytoskeleton disruption; (4) metabolic changes that leads to low levels of ATP; and (5) difference of potential in membrane permeability that cause increases in intracellular levels of Na^+, H^+ and Ca^{2+} (16).

(b) Hyperthermia can cause decrease in viscosity of the plasma membrane and also in lipid bilayers (17).These changes affect the alteration in membrane transport functions which is directly connected to the activities of the ATP-dependent sodium-potassium pump (18).

(c) Hyperthermia causes increase oxidative stress. Increase in reactive oxygen species (ROS) occurs during both non-lethal (40°C) and lethal (≥42°C) temperatures which causes intracellular oxidative damages to nucleic acids, proteins and lipids (19, 20). Hyperthermia increases the formation of high levels of hydrogen peroxide.

(d) High levels of hydrogen peroxide (H_2O_2) formed due to hyperthermia also perturbs with the normal intracellular mitochondrial membrane potential (21-23). This mechanism leads to protein degradation followed by inhibition of cellular proliferation, ultimately leading to cellular death by apoptosis (non-lethal temperature) or necrosis (lethal temperature).

2. Discussion

Recently, authors have also focused on the fact that hyperthermia degrades the BRCA2- protein, which is one of the essential components in DNA double strand break repair via the HR pathway (3). BRCA2 helps in loading RAD51 onto DNA breaks and thereby helps in the repair process. Degradation of BRCA2 by hyperthermia causes abnormal localization of RAD51 which, in turn, attenuates the process of DNA repair via HR (3). Hyperthermia-mediated BRCA2 degradation creates definite opportunities to increase the efficacy in treatment, because it induces a localized environment in HR-deficiency (14). Therefore, this area can be potentially explored by new treatment modalities that may reduce the risk of side-effects in cancer treatment like combination of hyperthermia with new classes of indirect double-strand break inducing agents called PARP-inhibitors (24-26). Several authors have also found high mortality rate in ovarian cancer as compared to other solid tumors (27). A number of studies suggested that ovarian cancer is generally 50% HR competent and 50% HR deficient, but researchers also focused on the fact that several HR competent tumors is found to be chemo-resistant (28). So, if hyperthermia is treated in combination with PARP inhibitors, the lethality will be more pronounced as compared to other treatment regimens (29).

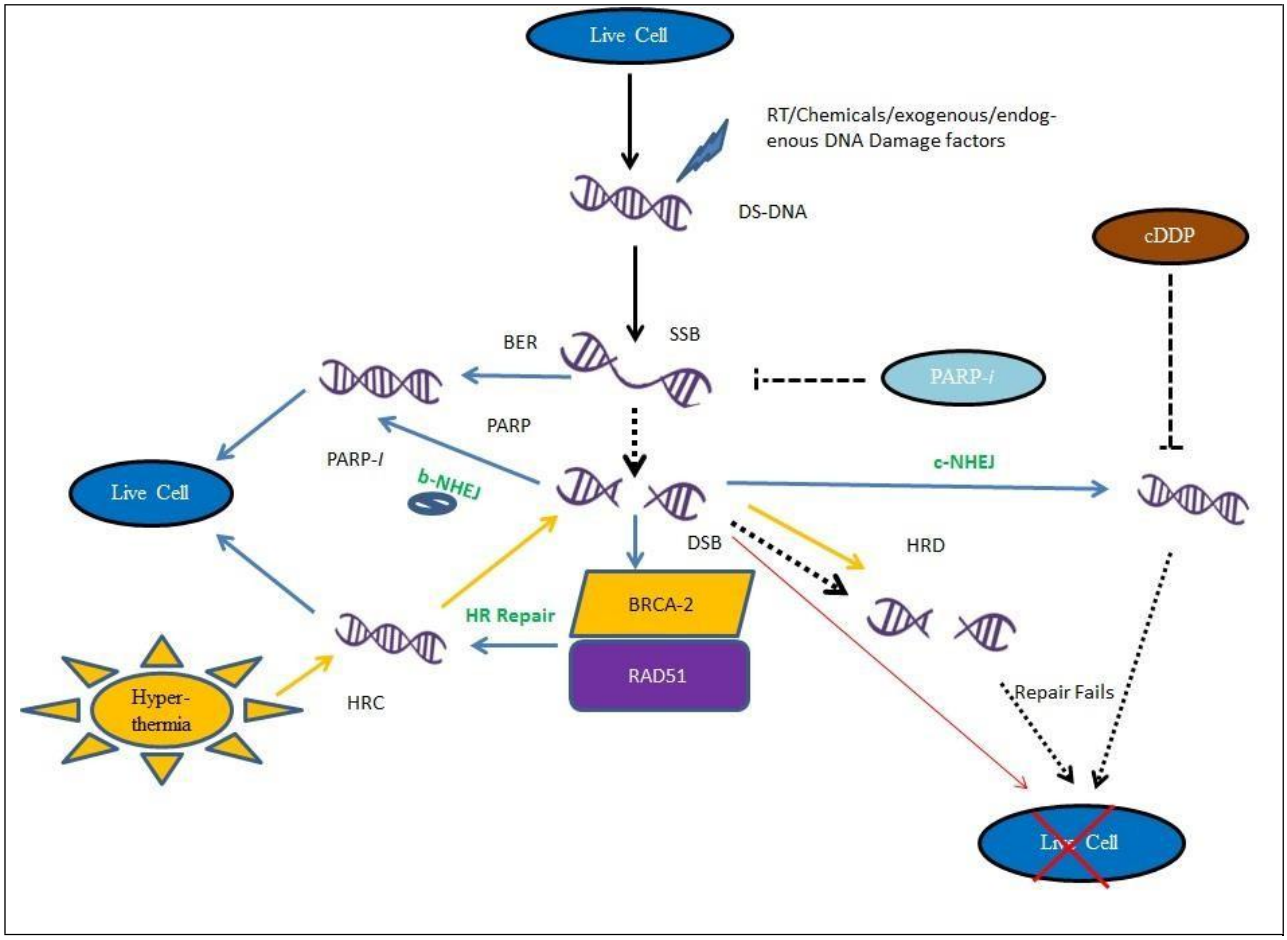

Figure 1: The graphical representation of this review.

The overall summary of this review is sketched in Figure 1. ds-DNA in live cell gets damaged with various chemical or radio frequency or any other endogenous factors. This generates the break in the ss-DNA which is generally repaired by the BER pathway with the help of the activities of PARP protein along with other DNA repair enzymes (28). If PARP inhibitors are used, then ss-DNA breaks will be converted to ds DNA break in next cell cycle which is repaired in the S phase with the help of BRCA2 with RAD51 at the end of the homologous recombination repair pathway. Now if this HR pathway is not efficient then the cell will die but sometimes NHEJ, an error porn repair process is activated throughout the cell cycle which repairs the DNA damage and cells become viable. Some studies have shown that cisplatin can block this classical NHEJ pathway but still back up/alternative NHEJ pathway may gets activated and cell may get repaired. This backup NHEJ repair process also may get blocked by using PARP inhibitors (14, 19, 29-32). Cells that are HR deficient will not survive but if the cells are HR proficient, it needs to be deactivated in order to achieve better survival rate in cancer patients. To attenuate these HR proficient cells, hyperthermia can be the best choice as it acts as a physical agent with less side effects. Hyperthermia degrades the BRCA2 protein including several other important factors which requires HR repair pathways for their survival. So, cisplatin in combination with PARP inhibitor and hyperthermia can be the best choice of treatment that follows HR competent DNA repair pathway to achieve the better survival rate.

An approach by Kanaar et al showed that when PARPi is used in combination with cisplatin and hyperthermia therapy, it could slightly increase the overall treatment efficacy, which potentially allowed use of cisplatin at reduced concentration (31). Also, different strategies allowed potentiation of the cytotoxic and sensitizing effects of hyperthermia which can lead to improved therapy outcomes via multiple paths one of which is inducing stronger cytotoxicity.

3. Conflict of Interest

The authors show no conflict of interest.

4. List of Abbreviations

Ultraviolet; UV, single strand breaks; SSBs, double strand breaks; DSBs, nucleotide excision repair; NER, mismatch repair; MMR, base excision repair; BER, non-homologus end joining; NHEJ, homologous recombination; HR, poly (ADP-ribose) polymerase; PARP, homologus recombination repair; HRR, Replication Protein A; RPA, single-stranded DNA; ssDNA, DNA binding domain; DBD, automodification domain; AMD, C-terminal catalytic domain; CD, poly (ADPribose)glycohydrolase; PARG, poly(ADP-ribose); pADPr, DNA ligase III; Lig III, DNA polymerase β; pol β, X-ray complementary gene1; XRCC1, 3-amino benzamide ; 3AB, dimethyl sulphate; DMS, reactive oxygen species; ROS, reduced glutathione; GSH, hydrogen peroxide; H_2O_2.

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