

The Effect Of Cellular Stress On DNA Repair Targeted Ovarian Cancer Therapy

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Abstract

Approximately more than half of the high-score serious cancers of epithelial ovarian suffer changes in the gene of homologous recombination, while the remaining make changes in other repairs of DNA ways at excessive rate or frequency (Somyajit et al., 2010). Such gene alterations that are cancer-specific can consult a selective amount of responsiveness to harmful agents of DNA like carboplatin and cisplatin, etoposide, topotecan, doxorubicin and gemcitabine. PARP inhibitors that in the near past have been frequently used by the FDA in order to treat advanced cancer caused in the ovaries. This also works as a DNA destructive agent by prompting complexes of PARP-DNA. The DNA destructive agents make many other kinds of DNA that need specific DNA in order to repair DNA genes. This usually convinces duplication fork slowing, which is also known as stress. Duplication of stress generally produces or triggers DNA repairs in the form of checkpoint proteins; these proteins further prevent DNA damage. Therefore, the targeting of DNA repair genes or the checkpoints of DNA repair argue the anti-tumour activity of agents responsible for DNA injury. The research described the balanced approach to using the DNA repair and checkpoint of DNA repairs acts as a sole agent. This research has also presented the strategies for combining these agents with damaging DNA agents in the therapy of ovarian cancer based on some specific gene alteration. There is an accruing indication that it may certainly be achieved and that cellular stress can be minimized by curing with select pharmacological agents. The ethics for this new approach to cure cancer, as well as representative ER stress-aggravating compounds, will be presented in this review.

Study Aim

The aim of this study is to compare the effects of the anti-cancer drug Olaparib in two human cancer cell lines with normal and aberrant expression of the DNA repair gene BRCA1.

Study Objectives

The objectives of this study include:

To gain experience in working with human tissue culture, which includes:

- Handling of adherent cancer cell lines;

- Maintaining stable cell lines over-expressing DNA repair proteins of the interest.

Asses the molecular effect of the anti-cancer drugs, Olaparib and Cisplatin on:

- Genome stability;
- Cell proliferation;
- Cell apoptosis.

Analyze chromosomal composition in cells with different BRCA status after treatment with anticancer drugs Olaparib and Cisplatin.

Introduction

The maintenance of genome integrity is the most important commission in the life of a cell or entire organism. The integrity of the chromosomal or gene level allows for normal functioning and survival. Any DNA lesions lead to cell cycle arrest, followed by either successful DNA repair or mutation-triggered apoptosis.

However, a specific set of genetic mutations permits the avoidance of cell cycle arrest and overcoming all checkpoints, leading to uncontrollable cellular growth and resulting in cancerous transformation.

Cancer has become a leading life-threatening disease in the modern world. According to the GLOBOCAN study, over 14 million new cancer cases and over 8 million cancer-related deaths were registered in 2012 worldwide (Ferlay et al., 2015). Currently, 27 major types of cancer are described. Among those, lung cancers account for 1.82 million, breast cancers for 1.67 million and colorectal cancers for 1.36 million commonly diagnosed cases (Ferlay et al., 2015). Lung cancers are the main type of cancer linked to mortality. The treatment of breast cancer has advanced in recent years, which allows for significantly extended disease-free survival.

Continuous cellular stress is a major basis of deteriorating genome integrity, which compromises the extracellular environment and causes damage to DNA, RNA and protein structure and functions (Poljsak & Milisav, 2012). There are many stressors, and different kinds of them infer different cellular responses, impairments and repair mechanisms. Most of the stressors are triggering damage to DNA structure. Persistent stress generates mutations and, as a result, increases susceptibility to cancer and ageing (Poljsak & Milisav, 2012). Excessive stress-related damage results in the induction of autophagy and apoptosis (Poljsak & Milisav, 2012).

Type of mutations and DNA repair machinery

DNA damage is generally classified by its source as oxidative, chemical, hydrolytic and radiation-induced. The type of damage has corresponding structural features such as deamination of bases, base conversions, formations of DNA adducts, and DNA strand breaks. Each type of DNA damage requires its own mechanism for the repair. Disturbances in DNA repair pathways can predispose cells to accumulating damage-specific DNA mutations.

The most serious DNA injuries are single- and double-strand breaks of the DNA chain. They are recognized as the main drivers of carcinogenic processes (Aparicio, Baer, & Gautier, 2014). Single-strand break (SSB), if left unrepaired, can invade the intact double helix, insert a single-stranded chain of DNA into it and create a triple helix structure. This often results in the formation of DNA double-strand break (DSB). As opposed to most of the types of DNA damage that could be repaired with little homology required, DNA strand breaks can be repaired by homologous recombination (HR) without sequence changes only with a large homological DNA stretch used as a template. This repair can be performed only at specific cell phase, S phase, when sister chromatids are present and provide that homology. At the G1, G2, or G0 cell cycle phases, the repair could also be executed but mainly by non-homologous end joining (NHEJ), which does not require extensive homology and is therefore prone to errors (Kelley, Logsdon, & Fishel, 2014).

DNA strand breaks and their repair

The scheme of the DNA repair pathway driven by homologous recombination is shown in Fig. 1. As found previously, PARP activation is the earliest response to a single or double-strand DNA break. The protein PARP facilitates several enzymatic reactions at the site of DNA damage, keeping the broken ends together and relaxing the surrounding chromatin, thus making the site available for the repair of protein complexes (Yan et al., 2013). Following PARP activation, the ATM protein is recruited to the site. This protein determines which repair pathway will be activated: NHEJ or HR (Aparicio et al., 2014). There is no clear understanding of the mechanism of pathway choice. However, it is known that HR requires the activity of BRCA 1 and 2 proteins (Zhang & Powell, 2005), as well as the activity of PCNA (Hasan, Hassa, Imhof, & Hottiger, 2001).



Figure 1. Schematic view of homologous recombination of DNA strand breaks (Yan et al., 2013)

BRCA1

The BRCA1 gene is mapped on chromosome 17q21. The gene encodes a 1,863 amino acid protein containing several zinc-finger domains close to the N terminus of the protein. A high level of BRCA1 expression is found in breast, ovary and testes tissues.

Mutations in BRCA1 and BRCA2 genes are often detected in familial breast and ovarian cancers. BRCA1 mutations are prevalent in hereditary breast cancer, accounting for 45 per cent of clinically relevant findings, and BRCA1 mutations are even more prevalent in families with hereditary breast and ovarian cancers, together accounting for 80 per cent of the cases (Zhang & Powell, 2005). Mutations in the BRCA1 gene are associated with breast and ovarian cancers and have multifactorial or autosomal dominant inheritance.

In addition, the BRCA1 protein expression is significantly compromised or absent in sporadic breast malignancies (Zhang & Powell, 2005). The cellular function of the BRCA1 protein is described as a critical component in DNA repair and cell cycle checkpoint control (Wang et al., 2009). Another direct function of BRCA1 protein is a modulation of chromatin structure, namely chromatin decondensation, which is required for the control of transcription (Bochar et al., 2000; Ye et al., 2001).

PARP1

The poly(ADP-ribose) polymerase (PARP) is a gene mapped on chromosome 1q42.12. The protein is nuclear and co-localized with nucleosomal core histones. The enzyme catalyzes the transfer of ADP-ribose to a number of nuclear proteins. The activity of the enzyme is induced by the presence of DNA single-strand breaks, which provide a substrate for the PARP enzymatic activity (Alkhatib et al., 1987). ADP-ribosylation is a DNA break-dependent protein modification which serves as a signal for DNA repair needs. There is a positive correlation between the PARP activity level of individuals and their life span, which probably reflects the efficacy of genome integrity maintenance (Grube & Burkle, 1992). Immunostaining showed that PARP proteins are co-localized with mitotic spindle, and hydrolytic breakage of the protein resulted in rapid spindle disassembly, which blocked the correct chromosomal segregation during cell division (Chang, Jacobson, & Mitchison, 2004). PARP is required for gamma-H2AX and Rad51 foci formation at the site of DNA break, triggering DNA repair and recruitment of ATM to the site of DNA damage (Bryant et al., 2005). The absence of PARP at the DNA break site or its inhibition causes collapse of replication forks via the broken DNA end intervention arresting the cells in S phase (Bryant et al., 2005). Moreover, BRCA-deficient cells are very sensitive to the PARP enzymatic activity levels. The impaired function of PARP in BRCA-deficient cells leads to cycle arrest and cell death (Bryant et al., 2005; Farmer et al., 2005).

DNA repair pathways in targeted anticancer therapy

The observed effect of PARP inhibition on apoptosis in the BRCA deficient cells led to the idea of targeted anticancer therapy (Fig. 2). Monotherapy by PARP inhibitors could be used to treat BRCA deficient tumours (Bryant et al., 2005; Farmer et al., 2005; Fong et al., 2009). Such an approach ensures lower toxicity for the patients. The improved toxicity profile originates from the fact that only cells with the lost BRCA activity are subject to PARP inhibitor-induced cell death (Fong et al., 2009). The mechanism of PARP-targeted antitumor therapy consists of restricting the DNA break repair in the

cells already deficient in homologous recombination due to BRCA dysfunction. This leads to the accumulation of DNA damage, cell cycle arrest and cell death triggered by these factors (Helleday, 2011). As demonstrated *in vitro* and by using xenografts, BRCA aberrant cells are drastically more sensitive to the PARP inhibitors compared to the normal cells (Fong et al., 2009).



Figure 2. PARP-targeted cancer therapy in BRCA deficient cells

Currently, several PARP inhibitors, including olaparib, veliparib, olaparib, olaparib and talazoparib, are tested for targeted anticancer therapy.

Olaparib

Nine clinical trials were conducted to prove the safety and efficacy of PARP-targeted therapy in BRCA-deficient breast cancers (Javle & Curtin, 2011). The most promising results were obtained with Olaparib. A phase 1 clinical trial on breast cancer patients carrying BRCA mutation demonstrated that Olaparib provides clinical benefits in 63 per cent of cases, with positive tumour marker responses and significant disease stabilization (Fong et al., 2009). In a phase 2 clinical trial on BRCA-deficient ovarian cancer patients, the efficacy and safety of Olaparib were also confirmed (Konecny & Kristeleit, 2016).

Cisplatin

Cisplatin is another widely used compound for anticancer chemotherapy. In many cases, patients develop reduced sensitivity to cisplatin (or even have it at the start). The latest investigation showed that the synergetic use of PARP inhibitor olaparib sensitizes cancer cells to cisplatin in cervical cancer patients (Wang et al., 2017). Cisplatin toxicity could be overcome via PARP1 regulation. This will possibly provide future direction for PARP-based approaches beyond BRCA-deficient cancers (Singh, Chauhan, & Kang, 2018; Xu et al., 2017).

Material and Methods

Cell lines

In the present study, two cancer cell lines were used: MCF7 cells derived from adenocarcinoma of the breast and HeLa Kyoto cells genetically modified to stably express the fused protein green fluorescent protein-tagged proliferating cell nuclear antigen (GFP-PCNA). Both cell lines have epithelial origin. However, the cell lines derived from different cancerous tissues: MCF7 was obtained from the metastatic side of a breast cancer patient, and HeLa was established from a cervical cancer specimen. The cell lines differ in BRCA1 status.

MCF7 is characterized by loss of heterozygosity at the BRCA1 locus, which is reflected in the reduction of the BRCA1 expression level (Elstrodt et al., 2006; Kachhap et al., 2001; Vissac et al., 2002). The karyotype of MCF7 cells has a modal chromosome number equal to 82 on average, ranging from 66 to 87 chromosomes per nucleus, which corresponds to hypertriploidy or hypotetraploidy. The cells carry multiple marker chromosomes, up to 34 marker chromosomes per cell. Approximately one-third of the cellular population has markers in their nuclei. In the vast majority of cells with marker chromosomes, those chromosomes are present as a single large submetacentric marker chromosome, three large subtelocentric marker chromosomes and a set of smaller marker chromosomes (Rondon-Lagos et al., 2014). Chromosome 20 is deleted. Despite pronounced transformation and chromosomal abnormality, MCF7 cells still feature characteristics of differentiated mammary epithelium. MCF7 cells are estrogen-sensitive and express WNT oncogene.

In contrast, the HeLa Kyoto cell line carries two normal, not mutated BRCA1 genes that have not changed their expression levels compared to healthy cervical epithelium (Landry et al., 2013; Vissac et al., 2002). The karyotype of HeLa cells shows multiple rearrangements and changed ploidy with a modal chromosome number of 82, ranging from 70 to 164 chromosomes per cell. As shown by G-banding, almost a hundred per cent of cells carry multiple marker chromosomes originating from the fusion between chromosomes 1 and 3, 3 and 5, and 11 and 19 (Chen, 1988).

The GFP-Flag-PCNA-carrying HeLa-Kyoto cells are a great instrument for studying cell cycles by microscopy. Previous studies demonstrated that the PCNA gene expression is clearly associated with the S-phase of the cell cycle. It was previously shown that PCNA expression marks the beginning of the S-phase, and the product becomes undetectable shortly before the cells enter the next phase of the cell cycle (Celis et al., 1986; Kurki et al., 1986).

DNA-damaging reagents

In the present study, two anticancer drugs acting via PARP1 were used: Olaparib and Cisplatin (Sigma-Aldrich, USA).

Olaparib (SIGMA-Aldrich)

Olaparib is the name for 4-[4-Fluoro-3-[(4-methoxypiperidin-1-yl) carbonyl] benzyl] phthalates-1 (2H)-1, the organic compound with a molecular weight of 395.43 Da and empirical formula $C_{22}H_{22}FN_3O_3$ (Fig. 3).



Figure 3. Molecular structure of Olaparib.

Olaparib is a PARP inhibitor that induces lethality in BRCA1/2 deficient cells, which carry either homologous deleterious BRCA mutations or compound heterozygotes for those genes (Audeh et al.,

2010). Olaparib demonstrates an antiproliferative effect in cells with BRCA1 and BRCA2 mutations and cells with BRCA1 allelic loss. In addition, there is evidence of autophagy/mitophagy induction by Olaparib in BRCA-deficient cells (Arun et al., 2015).

Cisplatin (SIGMA-Aldrich)

Cisplatin is the name for cis-diammineplatinum (II) dichloride, a chemical with a molecular weight of 300.05 Da and linear formula $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$. PARP1 is one of the targets for cisplatin and its prodrugs (Xu et al., 2017). Cisplatin reacts with water and DNA molecules and forms bulky adducts with DNA, thus preventing replication. The consequent interaction of cisplatin and DNA complex with PARP1 protein results in cell death (Michels et al., 2013).

Cell culture

The cells were grown in DMEM media containing 10% FCS and supplied with L-glutamine and antibiotics according to the ACCT repository instructions. The cells were split twice per week in a 1 to 5 ratio using a trypsinization procedure (Surendranath, 2014)

Cell proliferation and viability test

The Resuzurin Viability Kit (Biotium Inc., USA) was used to determine cell proliferation and viability. All steps were performed according to the manufacturer's protocol. The data was obtained by measuring the absorbance at 570 nm and 600 nm using an absorbance microplate reader for colourimetric detection and by measuring fluorescence with excitation/emission at 570/585 nm using a fluorescence microplate reader for fluorescent-based detection. Absorbance values were adjusted to the background level and plotted against the density of the plated cell. The optimal values were identified for future use.

Metaphase Spreads Preparation:

Metaphase spreads were prepared using standard Colcemid (Life Technologies) arrest procedure followed by hypotonic treatment and standard methanol/acetic acid fixation procedures (Surendranath, 2015)

Olaparib and Cisplatin treatment

To study the effect of Olaparib and Cisplatin, MCF7 and HeLa Kyoto cells were plated on the coverslips and placed into six-well culture plates or directly into 12 well culture plates and grown to 70% confluence. The culture media was removed, and cells were washed three times with 1XPBS to

remove any traces of FCS. Then, DMEM with 0.2% FCS was added for 16 hours to synchronize the cells. The synchronization level of cell cultures was assessed by live fluorescent microscopy, detecting the green fluorescence produced by the GFP-PCNA fusion construct product in HeLa Kyoto cells.

After the starvation step, low serum media was replaced with complete DMEM media containing 10% FCS and supplemented with either Olaparib or Cisplatin at different concentrations. Olaparib concentrations were 0, 100, 200 and 300 nM, and Cisplatin concentrations were 0, 0.15, 0.3, 0.6, 1.25, 2.5, 5, 10, 20, 30 and 40 mM. The cells were incubated at 37°C for 48 hours and then subjected to live microscopy, cell proliferation assay, cell viability assay or Giemsa staining.

To perform this procedure, compare the effects of the definite anti-cancer drug Olaparib in two separate human ovarian cancer cell lines, in the BRCA wild type and in the BRCA mutant. Following are the procedures that were applied

- Genome stability
- Cell proliferation
- Cell apoptosis

Results



Figure: Images of HeLa Kyoto GFP-PCNA cells treated for 48 hours with different concentrations of Olaparib.



Figure: Images of HeLa Kyoto GFP-PCNA cell in visible and fluorescent light.



Figure: HeLa Kyoto PCNA-GFP were plated in multi-well dishes and treated with Olaparib with increasing concentration for a 48-hour period and viewed under a bright field microscope. Furthermore, these were fixed, and resazurin was added.

Discussion

It is to visualize the duplication or replication in the cells. The CHO cells that stably express human PCNA were generated and tagged with GFP. Some other tests showed that GFP-hPCNA with the anti-PCNA antibodies had a similar level of expression.

Duplication of the genome of mammals starts at several thousands of origins that are generally turned on at diverse times during its phase. However, a specific set of genetic mutations permits the avoidance of cell cycle arrest and overcoming all checkpoints, leading to uncontrollable cellular growth and resulting in cancerous transformation.

Induced DNA duplication stress is the key to the instability of chromosomes in cancer in humans. This is supposed to play a role in the formation of oncogenesis. It is imperative, hence we not only try to understand the cause of stress, but we also try to cover the path ways that are used by the cells to show its potential effects oncogenically. When it is challenged by suplication stress, the program of duplication that is taking place at CFSs is often assigned to such an extent that it continues to synthesise DNA even after the point where the cell has managed to enter the initial phase of mitosis. Recent research has found that it is not conventional to synthesise DNA.

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