Asian Oncology Research Journal

1(1): 1-6, 2018; Article no.AORJ.38836

Determination of the Location of Uracil-DNA Glycosylase (UDG) Protein in Lungs Cancer Cells

A. A. Minjibir[1] and M. Ali[2*]

[1]Department of Pharmaceutical Technology, School of Technology, Kano, Nigeria.
[2]Department of Microbiology, Kano University of Science and Technology, Wudil, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author AAM designed the study, performed the experiment, wrote the protocol and wrote the first draft of the manuscript. Author MA managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AORJ/2018/38836
Editor(s):
(1) Omid Iravani, Department of Anatomy, National University of Singapore, Yong Loo Lin School of Medicine, Singapore.
Reviewers:
(1) Janani Kumar, University of Texas MD Anderson Cancer Center, USA.
(2) Michel Mansur Machado, Universidade Federal do Pampa, Brasil.
Complete Peer review History: http://www.sciencedomain.org/review-history/22922

Original Research Article

Received 26th October 2017
Accepted 5th January 218
Published 29th January 2018

ABSTRACT

Background: In every 24 hours, about 100-500 Cytosines undergo unprepared deamination in a particular cell. The deamination of cytosine to uracil is one of the major pro-mutagenic events in DNA causing G: C→A: T transitional mutations if not repaired before replication take place. Uracil-DNA Glycosylase (UDG) is one of the major proteins that coordinate multiple cellular activities in the cell. However, its positions in the cell determine the type of activity it controls. Repair of uracil-DNA is achieved in base-excision pathway initiated by UDG.
Aim: The research was aimed to determine the location of *UDG* in lungs cancer (SW480*)* cells.
Methodology: To determine the location of *UDG* protein in lungs cancer SW480 cells, the cells were treated with 100 mU Bleomycin (BLM) and 100ug 5-Flourourecil (5-FU).
Results: The FITES florescence photograph of the cells shows that UDG protein is localizing in the cytoplasm as seen by a glowing green colour of the tagged antibody around the cell's nucleus in the cytoplasm in both treated and untreated cells.
Conclusion: The DAPI florescence photograph shows a dark central image with no glowing of anti-mouse antibody indicating the absence of the protein in the nucleus.

**Corresponding author: E-mail: alimuhd4real@gmail.com;*

Keywords: Uracil DNA glycosylase; bleomycin; 5- fluorouracil; genes; antigens.

1. INTRODUCTION

Deamination of cytosine to uracil is one of the major pro-mutagenic events in DNA, causing G: C→A: T transition mutations if not repaired before replication take place. Repair of uracil-DNA is achieved in a base-excision pathway initiated by uracil-DNA glycosylase (UDG) [1]. Uracil (U) arises in DNA either by a disincorporation of dUTP through deamination of cytosine (C) or during DNA synthesis (forming U: A pairs) inside the DNA duplex (resulting in U: G mispair). Apart from cytosine, mammalian cells also have 5-methylcytosine (MC) and 5-hydroxymethylcytosine (HMC) [2]. In every 24 hours, about 100-500 cytosines undergo unprepared deamination in a particular cell [3]. Therefore, if left unrepaired, cytosine deamination is the key cause of spontaneous mutation [4]. DNA glycosylases *UNG and SMUG1* belong to the same protein super family that remove uracil from DNA [5], and they are coordinating the first phases of base-excision repair (BER) by discrete mechanisms. The less efficient *SUNG2* significantly repair a non-replicating cytosine deamination (U: G), whereas *UNG2* superficially and accurately repair uracil (U: G and U: A) in replicating DNA.

Based excision repair (BER) pathway begins with the excision of the uracil base from the DNA by the enzyme uracil-DNA glycosylase (*UDG*) and creating abasic site or a purinic/apyrimidinic (AP)-site. However, increase in the levels of dUTP by 5FU can elevate the probability of deoxypuridine being incorporated into DNA again and require the triple *apn1, apn2* and *tdp1* knockout to confer sensitivity [6]. Whether the repair of oxidized abasic site or the removal of unsaturated abasic residues is generated by AP lyase, its activity requires APE1. Moreover, APE1 is also involved in the conversion of the 3′-PG (at oxidative breaks) to 3′-OH prior to gap filling/ligation process of DNA repair [7].

1.1 DNA Damage Induced Repair

Most of the commonly used chemotherapeutic cytotoxic compounds or ionising radiations (IR) causes' high level of DNA damages, that activates cell cycle check points leading to cell cycle block and/or cell death [8]. Double Stand DNA Breaks (DSDBs) is considered as one of the most fatal form of DNA damage which can be caused by agents such as radiomimetic chemicals (bleomycin and neocarzinostatin), antimetabolites 5-fluorouracil (5FU), IR, topoisomerase inhibitors (camptothecin), and chemicals that generate reactive oxygen species [8,9]. However, acquired resistance of tumours to some of these chemotherapeutics, e.g. 5FU [10] and bleomycin [11], have caused therapeutic failures. DNA repair following chemotherapy insult is often associated with such tumour resistance. The understanding of how the cells respond to the DNA damage causing agents or manage to repair the lethal lesions becomes a key to increasing the efficacy of the anticancer agents. In this study, the focus will be on understanding action of two anticancer agents: 5FU and bleomycin on the location of UDG protein in mammalian lungs cancer cells with aim of ascertaining whether UDG can repair DNA damage caused by these agents.

1.2 5FU-Induced DNA Damage

5FU is one of the widely used antimetabolite drug for treating malignancies including colorectal, breast, stomach, pancreatic, oesophageal, head and neck cancers. 5FU has been reported to work either by inhibiting vital biosynthesis processes or by integrating themselves into DNA and RNA, and hence preventing normal function. Evidence suggests a more complex mechanism for 5FU involving pyrimidine nucleotide balances, DNA repair processes and disruption in RNA metabolism [12,13].

1.3 Mechanism of Action

5-FU acts in several ways, one of which is thymidylate synthase (TS) inhibitor. In this case it interrupted the action of thymidylate synthase (TS) thereby blocking the synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication. The thymidylate synthase (TS) methylates deoxyuridine monophosphate (dUMP) to form thymidine monophosphate (dTMP) during DNA replication. Administration of 5-FU cause's scarcity in dTMP and as result, the dividing cancerous cells will undergo cell death via thymineless death [12]. Calcium folinate provides an exogenous source of reduced folinates and hence stabilizes the 5-FU-TS complex, thereby enhancing 5-FU's cytotoxicity [14].

1.4 5FU Toxicity and Damage to RNA and DNA

5FU is believed to interfere with nucleic acid structure and function by directly incorporating fluoronucleotides into DNA and RNA, and through inhibition of thymidylate synthase by active metabolite generated by 5FU, which leads to imbalance in the nucleotide pool [13].

1.5 Bleomycin-Induced DNA Damage

The types of lesions induced by bleomycin are dependent on the oxygenation conditions. In the presence of oxygen, bleomycin produces primarily DSDB, such as 3′-PG, whereas in the absence of oxygen it generates an oxidised AP-site. Radiomimetic drugs and IR are two of the anticancer treatments that induces tumour killing via DNA strand breaks. Bleomycin, a radiomimetic glycopeptide antibiotic, is used in the treatment of Hodgkin's lymphoma, non-Hodgkin's lymphoma, testicular cancer and cancers of head and neck. Bleomycin is less heavily used, and often causes impaired lung functions as a result of lipid peroxidation [11]. Bleomycin is believed to induce DNA damage similar to that of IR but different to 5FU. IR generates numerous types of damaged bases, abasic sites and other fragmentary products in addition to ss-breaks with 3′-phosphoglycolate (3′-PG) esters [7]. Bleomycin is reported to generate DNA base loss and cause ss- and ds-DNA damage in the presence of Fe (II) and oxygen [15]. Extraction of a hydrogen molecule from deoxyribose and formation of free radical is believed to enable Bleomycin-Fe (II)-O2 complex to break the DNA molecule [16]. This complex is also reported to cleave yeast tRNA, signifying that bleomycin oxidises RNA and DNA [17]. Furthermore, the redox status of a given cell type is stated to influence the kind of lesions that bleomycin generates. Under low oxygen level, bleomycin forms primarily AP-sites while in the presence of oxygen, it produces DNA strand breaks [11].

2. MATERIALS AND METHODS

2.1 Determination of the Location of *UDG* Proteins in Cancer Cells

UDG is one of the major proteins that coordinate many cellular activities. However, its position in the cell determined the type of activity it controls. The location of *UDG* in cancer cells was determined using lungs cancer *(*SW480*)* cells. The cells were supplied by Dr. Steve Safrany (Department of Pharmacology, University of Wolverhampton, UK). Six wells were prepared containing cells planted on microscopic slides. Two wells were treated with l00µg and l00mU of 5-FU and Bleomycin respectively, and the other two were left untreated as control. After one hour, the drugs were drained and the cells were washed with 2 ml of acetone followed by addition of blocking agent (3% BSA in PBS) while in ice and allowed for 5 minutes. The blocking agent was drained and the cells were washed again with 2ml of acetone. 2ml of blocking agent was added and allowed to stand for 2 hours in refrigerator; the cells were washed with PBS (Gibco), and then fixed with 2ml of 50/50% v/v methanol/acetone while in ice for 5 minutes. This was followed with addition of 2ml of blocking agent. After two hours at room temperature, l.5ml of 1 in 2000 ml of primary antigens solution was added to cell in the well. The blocking agents were allowed to drain and placed in an incubator at 4°C for 18 hours. The antibody was drained, washed three times with the blocking agent and once with PBS. The cells were placed on rocker for 2 hours after treating with l.5ml of 1 in 200 (1:199) ml secondary 2° antigens as blocking agent. The slides were washed again with 2ml of the blocking agent after draining the antigens, viewed and photographed using fluorescence microscope.

3. RESULTS

The results of the FITC photograph of the cells treated with 100mU BLM and 100ug 5-FU and untreated cell is presented in Figs. 1 - 6. FITSE (showing cell cytoplasm) and DAPI (showing cell nucleus) cameras were used. The photograph of the cells shows that UDG protein is localizing in the cytoplasm. FITC photograph using FITES camera of the cells shows a glowing green colour around the cell's nucleus (in the cytoplasm) in both treated and untreated cells, which was the position of *UDG* glowed by the antibody.

4. DISCUSSION

5FU and bleomycin are examples of oldest chemotherapeutics used today in clinic. Although, both the drugs causes DSDB [8][9], 5FU does it by irreversibly inhibiting TS and causing imbalance in the deoxynucleotide pool whereas bleomycin causes oxidative DSDBs in the presence of free radicals and metal ions. Action of these two drugs on DNA results in intermediates such as AP-site and 3′-PG adducts

which when left unrepaired can be cytotoxic and mutagenic to cells. Whether acquired or innate, tumour cells have found ways to exploit cellular repair mechanisms to correct the lesions and gain resistance against damage causing agents. Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR) are two of the well characterized primary pathways of DSDB repair. HR leads to accurate repair of DSDBs whereas NHEJ is potentially mutagenic [18]. Higher eukaryotes employ both the repair mechanisms while HR-mediated repair is predominantly seen in yeast [19,20]. Irrespective of the origin, it is widely accepted that the AP-site and 3′-PG removal requires a BER downstream enzyme, APE1. APE2 exhibits a weak endonuclease activity but possess strong exonuclease and 3′-phosphodiesterase activity [21]. Correspondingly APE1 appears to be the common enzyme that brings together the repair mechanisms of 5FU and bleomycin. However, recent studies reported that there is

Fig. 1. (FITES BLM). The Fluorescence photograph of the lungs cancer SW480 cells treated with 100mU BLM using FITES camera showing the cytoplasm of the cell. The glowing green colour around the cell's nucleus indicate the position of UDG protein in the cell cytoplasm

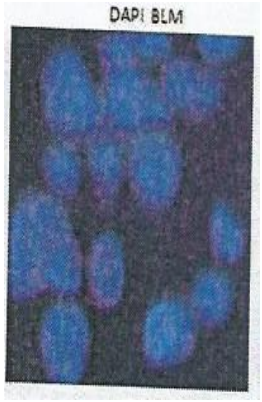

Fig. 2. (DAPI BLM). The Fluorescence photograph of the lungs cancer SW480 cells treated with 100mU BLM using DAPI camera showing the nucleus of the cell. The photograph indicated no UDG protein is found in the cell nucleus

Fig. 3. (FITSE 5-FU). The Fluorescence photograph of the lungs cancer SW480 cells treated with 100µm 5-FU using FITES camera showing the cytoplasm of the cell. The glowing green colour around the cell's nucleus indicate the position of UDG protein in the cell cytoplasm

Fig. 4. (DAPI 5-FU). The Fluorescence photograph of the lungs cancer SW480 cells treated with 100µm 5-FU using DAPI camera showing the nucleus of the cell. The photograph indicated no UDG protein is found in the cell nucleus

Fig. 5. (FITSE control). The Fluorescence photograph of the untreated lungs cancer SW480 cells using FITES camera showing the cytoplasm of the cell. The glowing green colour around the cell's nucleus indicate the position of UDG protein in the cell cytoplasm

Fig. 6. (DAPI control). The Fluorescence photograph of untreated lungs cancer SW480 cells using DAPI camera showing the nucleus of the cells. The photograph indicated no UDG protein is found in the cell nucleus

"division of labor between NHEJ and BER" in repair of AP-sites based on DNA sequence around the particular mismatch. However, this suggests that *UDG* protein is localizing by Bleomycin or 5-FU. DAPI photograph give the position of nucleus in a cell, and show a dark central portion indicating the absent of the protein in the nucleus. The results of this research suggested that, UDG protein in lungs cancer cells is cytoplasmic and is not localize or aggregate after treating SW480 cells with bleomycin or 5-FU. As a result of this, the gene can repair DNA lesion cause by the agents, as DNA decoding and replication is performed in the cytoplasm. The repairs activity of UDG gene and facilitation of cytotoxic activity of the agents (Blm and 5- FU) can be achieved by knocking out the gene.

5. CONCLUSION

From the result of this study, the FITES florescence photograph of the cells shows that UDG protein is localized in the cytoplasm as seen by a glowing green colour of the tagged antibody around the lungs cancer cell's nucleus both treated and untreated cells. It is recommended that; further research should be undertaken to find out how UDG repairs activity can be halt at the period of treatment.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/22922