

Original Research Article

DETERMINATION OF THE LOCATION OF URACIL-DNA GLYCOSYLASE (UDG) PROTEIN IN CANCER CELLS

ABSTRACT

BACKGROUND: In every 24 hours, about 100-500 Cytosines undergo unplanned 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 transition mutations if not repaired before replication. Uracil-DNA Glycosylase (UDG) is one of the major proteins that coordinate many cellular activities. However, its positions in the cell determine the type of activity it controls. Repair of uracil-DNA is achieved in a base-excision pathway initiated by UDG.

AIM: In this research the location of *UDG* was determined in *SW480* cells.

METHODOLOGY: To determine the location of *UDG* protein in *SW 480* cells, the cells were treated with 100mU 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 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.

Keywords: *Uracil DNA Glycosylase; Bleomycin; 5- fluorouracil; Genes; and 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. Repair of uracil-DNA is achieved in a base-excision pathway initiated by uracil-DNA glycosylase (UDG) [1]. Uracil (U) can arise 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 cytosine undergo unplanned 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 and remove uracil from DNA [5], and they are coordinating the first phases of BER by discrete mechanisms. The less efficient *SUNG2* significantly repair a non-replicating cytosine deamination (U: G), whereas *UNG2* superficially and accurately repair of 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 a basic site or a purinic/aprimidinic (AP)-site. However, increase in the levels of dUTP by 5FU could elevate the probability of deoxypuridine being incorporated into DNA again and require the triple *apn1*, *apn2* and *tdp1* knockout to confer sensitivity [6]. Whether it is the repair of the oxidised abasic site or the removal of unsaturated abasic residues 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

Many of the commonly used chemotherapeutic cytotoxic compounds or ionising radiations (IR) cause high levels of DNA damage, that activate cell cycle checkpoints, leading to cell cycle arrest and/or cell death [8](Swift and Golsteyn, 2014).Double Stand DNA Breaks(DSDBs), which are considered as the most fatal form of DNA damage, 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 and/or manage to repair the lethal lesions becomes the key to increasing the efficacy of the anticancer agents. In this study, the focus will be on understanding action of two anticancer agents: 5FU (Fig. 1) and bleomycin (Fig. 2) on the

location of UDG protein in mammalian cells with aim of ascertaining whether UDG can repair DNA damage cause by these agents.

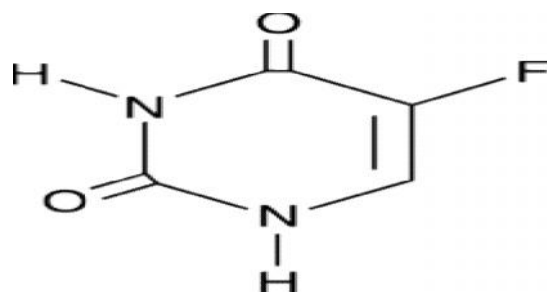


Figure 1: Structure of anti metabolite 5FU, adopted from chemspider.

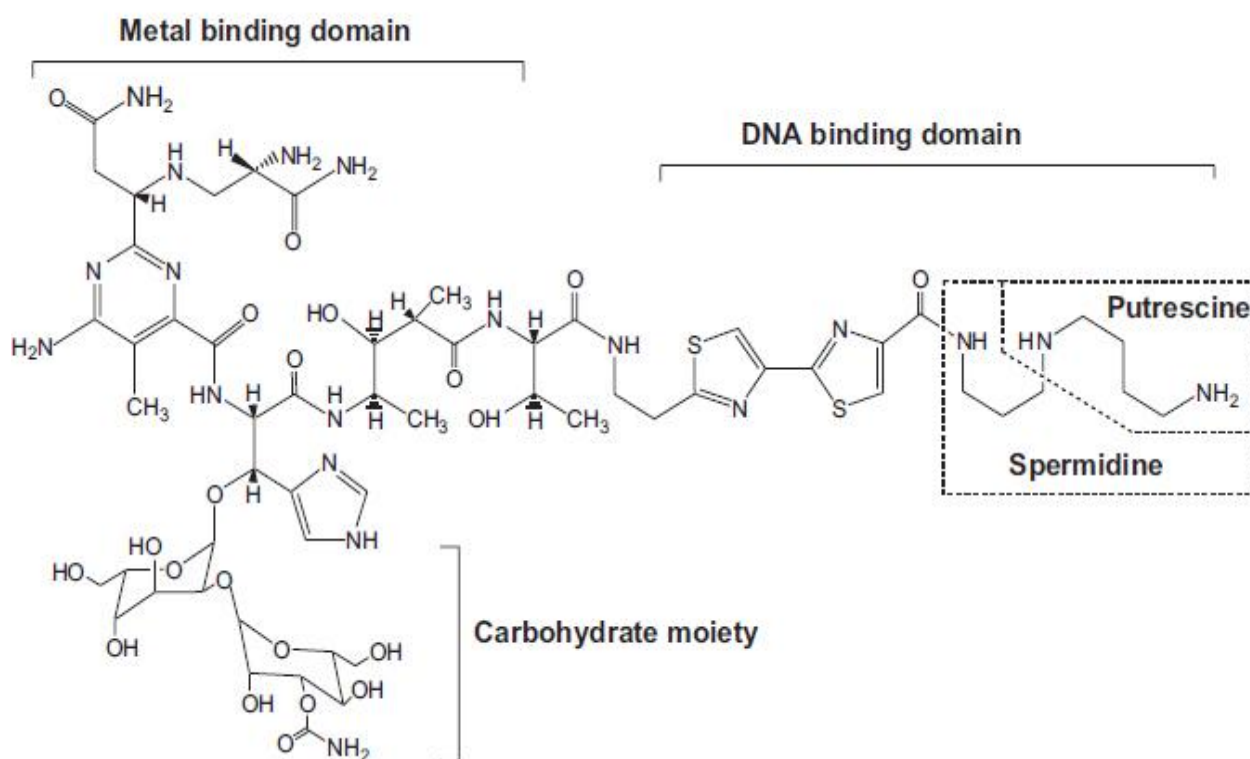


Figure 2: Chemical structure of the polyamine analogue, Bleomycin. Three regions are illustrated, and the polyamine moiety is required to intercalate with DNA figure 2 [12].

1.2 5FU-induced DNA damage

5FU is one of the widely used antimetabolite drug for treating malignancies including colorectal, breast, stomach, pancreatic, oesophageal and head and neck cancers. 5FU has been reported to work both by inhibiting vital biosynthesis processes and by integrating themselves into DNA and RNA, and 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, but principally as a thymidylate synthase (TS) inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication. Thymidylate synthase methylates deoxyuridine monophosphate (dUMP) to form thymidine monophosphate (dTMP). Administration of 5-FU causes scarcity in dTMP so rapidly dividing cancerous cells which undergo cell death via thymine less death [12]. Calcium folinate provides an exogenous source of reduced folinates and hence stabilises the 5-FU-TS complex, hence enhancing 5-FU's cytotoxicity Alvarez, P *et al.* [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 the 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, bleomycin generates largely 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 the deoxyribose and formation of a 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 as well as 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 levels, bleomycin forms primarily AP-sites while in the presence of oxygen, it produces largely DNA strand breaks [11].

2. MATERIAL AND METHOD

2.1 Determination of the location of *UDG* protein in cancer cells

UDG is a major protein that coordinate many cellular activities however, it positions in the cell determine the type of activity its control. The location of *UDG* in cancer cells was determined using *SW480* cells. The cells were supplied by Dr. Steve Safrany (Department of Pharmacology, University of Wolverhampton, UK). Six wells are prepared containing cells planted on microscopic slide. Two wells were each treated with 100µg and 100mU of 5-FU and Bleomycin respectively, and the other two were left untreated as control. After one hour, the drug is drained and the cells are washed with 2ml 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 are washed again with 2ml of acetone. 2ml of blocking agent was added again and allowed for 2 hours under refrigeration. The cells are washed with PBS (Gibco), and then fixed with 2ml of 50/50% v/v methanol/acetone while in ice for 5 minutes. This is followed with addition of 2ml of blocking agent. After two hours at room temperature, 1.5ml of 1 in 2000 (1:1999) ml primary ^{1°} antigens (mouse monoclonal antibody to *UDG*. Sicina VH0007374M1) to blocking agent solution was added to cell in the well after draining the blocking agent and placed in incubator at 4°C for 18 hours. The antibody was drained, washed three times with the blocking agent and once with PBS. The

cells are placed on rocker for 2 hours after treating with 1.5ml of 1 in 200 (1:199) ml secondary 2° antigens (FITC-labelled coat anti-mouse antibody Abcam ab6785) as blocking agent. The slides are 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 Figure 3. The photograph of the cells shows that UDG protein is localizing in the cytoplasm. FITC photograph of the cells shows a glowing green colour around the cell's nucleus in both treated and untreated cells, which is the position of UDG glowed by the antibody.

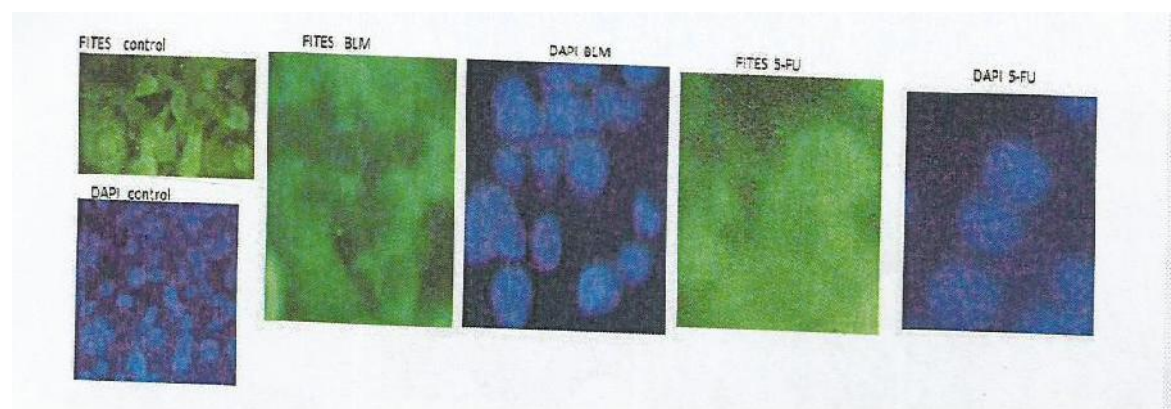


Figure 3: Fluorescence photograph of Sw480 cells treated with 100um Blm and 100µm 5-FU and the control.

4. DISCUSSION

5FU and bleomycin are the two oldest chemotherapeutics used today in the clinic. Although both the drug 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 or/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 the damage causing agents. NHEJ and 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 or/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 report that there is "division of labor between NHEJ and BER" in repair of AP-sites based on the 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 suggest that UDG protein is cytoplasmic and is not localize by both bleomycin and 5-FU. Being cytoplasmic and not localized by the agents, the gene can repair DNA lesion cause by this agents, as DNA decoding and replication is performed in the cytoplasm. The repairs activity of UDG gene and facilitation of the 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 localizing in the cytoplasm as seen by a glowing green colour of the tagged antibody around the cell's nucleus in both treated and untreated cells. It is recommended that; further research should be undertake to find out how UDG repairs activity can be halt at the period of treatment.

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