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LOCATION OF URACIL DNA GLYCOSYLASE (UDG) IN MAMMALIAN CELLS

Abdullahi Ahmed Minjibir
Department of Pharmaceutical Technology,
School of Technology, Kano State Polytechnic

ABSTRACT
Every day about 100-500 cytosine undergo unplanned deamination in a particular cell. 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 a major protein that coordinate many cellular activities however, it positions in the cell determine the type of activity its control. Repair of uracil-DNA is achieved in a base-excision pathway initiated by UDG. In this research the location of *UDG* was determined in*SW480* cells. 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). 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. However, the DAPI florescence photograph shows a dark central image with no glowing of antimouse antibody indicating the absence of the protein in the nucleus.

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

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 an uracil-DNA glycosylase (UDG) (Laurence H. Peal, 2000).Uracil (U) can arise in DNA either by a misincoporation of dUTP through deamination of cytosine (C) or during DNA synthesis (forming U: A pairs) inside the DNA duplex (resulting in U: G mispairs). Apart from cytosine, mammalian cells also have 5-methylcytosine (MC) and 5-hydroxymethylcytosine (HMC) (Dahl *et al,* 2011). Every day about 100-500 cytosine undergo unplanned deamination in a particular cell (Lindahl, 1993)). Therefore, if left unrepaired, cytosine deamination is th*e* key cause of spontaneous mutation (Kristin et al, 2012). DNA glycosylases *UNG andSMUG1* belong to the same protein superfamily and remove uracil from DNA. (Henrik *et al,* 2007), 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) (Ung1 in yeast) and creating an abasic site or apurinic/apyrimidinic (AP)-site. However, increase in the levels of dUTP by 5FU could elevate the probability of deoxyuridine being

incorporated into DNA again and require the triple *apn1apn2tdp1* knockout to confer sensitivity (Liu *et al.,* 2004). 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 (Fung and Demple, 2011).

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 (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 (Mahaney *et al.,* 2009; Swift and Golsteyn, 2014). However, acquired resistance of tumours to some of these chemotherapeutics, e.g. 5FU (Jette *et al.,* 2008) and bleomycin (Ramotar and Wang, 2003), 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.

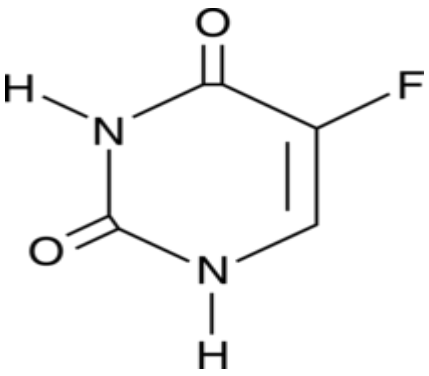

Figure 1: Structure of antimetabolite 5FU, adopted from chemspider.

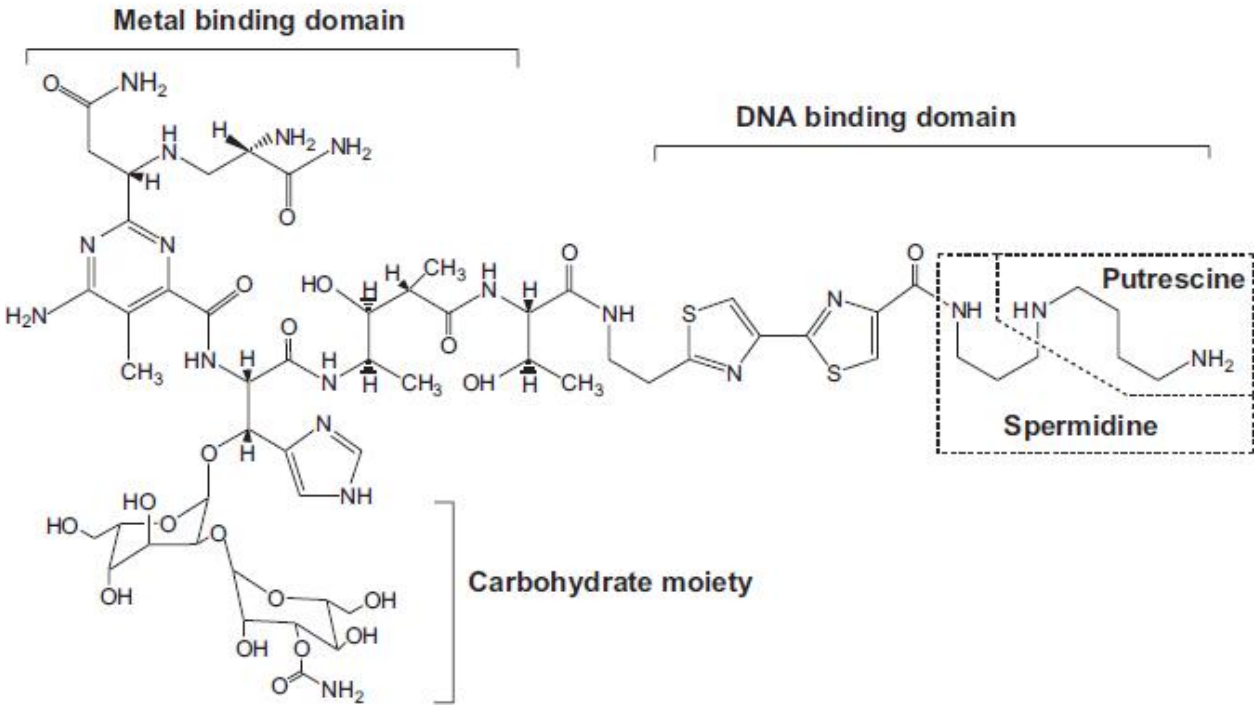

Figure 2 Chemical structure of the polyamine analogue, bleomycin
The three regions are illustrated, and the polyamine moiety is required to intercalate with DNA figure 2 (Romator, 2013).

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 (Longley *et al.*, 2003; Seiple *et al.*, 2006).

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 a scarcity in dTMP, so rapidly dividing cancerous cells undergo cell death via thymine less death Longley D. B. *et al* (2003). 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* (2012).

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 Seiple *et al.* (2006).

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 (Ramotar and Wang, 2003). 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 (Fung and Demple, 2011). Bleomycin is reported to generate DNA base loss and cause ss- and ds-DNA damage in the presence of Fe(II) and oxygen (Chen and Stubbe, 2005). Extraction of a hydrogen molecule from the deoxyribose and formation of a free radical is believed to enable Bleomycin-Fe(II)-O_2 complex to break the DNA molecule (Lim *et al.*, 1995). This complex is also reported to cleave yeast tRNA, signifying that bleomycin oxidises RNA and as well as DNA (Huttenhofer *et al.*, 1992). 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(Ramotar and Wang, 2003).

MATERIAL AND METHOD

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. 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 and 0.2% tween 20) while in ice and allowed for 5 minutes. The blocking agent was drained and the cells are washed again with 2ml of acetone. 2m1 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, l.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 l.5ml of 1 in 200 (1:199) ml secondary 2° antigens [FITC-labelledcoatanti-mouseantibodyAbcamab6785] as blocking agent. The slides are washed again with 2m1 of the blocking agent after draining the antigens, viewed and photographed using fluorescence microscope.

RESULT AND DISCUSSION

5FU and bleomycin are the two oldest chemotherapeutics used today in the clinic. Although both the drug causes DSDB (Mahaney *et al.,* 2009; Swift and Golsteyn, 2014), 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 lead to accurate repair of DSDBs whereas NHEJ is potentially mutagenic (Lieber *et al.,* 2003). Higher eukaryotes employ both the repair mechanisms while HR-mediated repair is predominantly seen in yeast (Ozenberger and Roeder, 1991; Pfeiffer *et al.,* 2004). 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 (Burkovics *et al.,* 2006). 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.

Location of UDG protein in sw480 cells

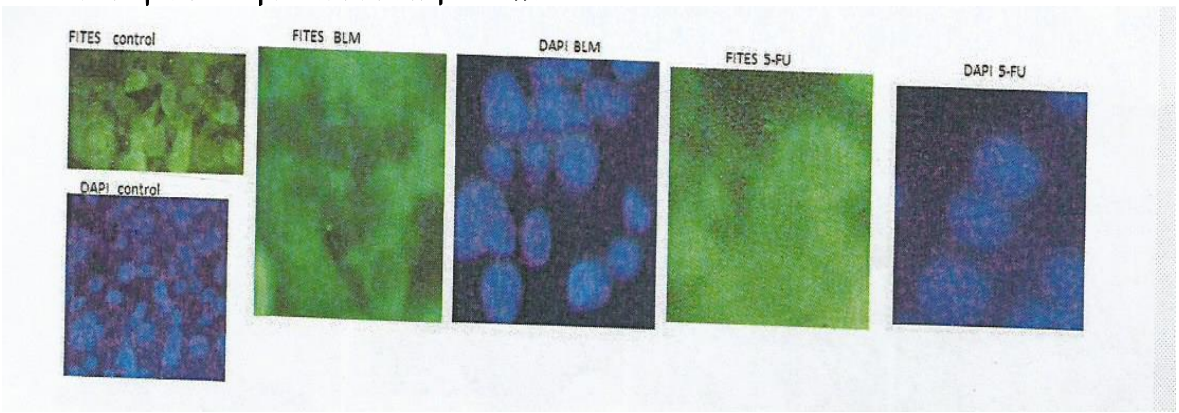

Figure 3 Fluorescence photograph of Sw480 cells treated with 100um Blm and 100μm 5-FU and the control.

To determine the location of *UDG* protein in *SW 480* cells, the cells were treated with 100mU BLM and 100ug 5-FU. The photograph of the cells shows that UDG protein is localizing in the cytoplasm figure 3. 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. 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 result 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. Conclusively, further research should be undertaking to find out how UDG repairs activity can be halt at the period of treatment.

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