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Analysis of the Repair of Topoisomerase II DNA Damage

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ABSTRACT: A large number of anti-cancer chemotherapeutics target DNA topoisomerases. Etoposide is a specific topoisomerase II poison that causes reversible double strand DNA breaks. This project analyses the repair of DNA damage induced by etoposide, a common anti-cancer chemotherapeutic. Through the comparison of two known DNA repair pathways, anti-cancer chemotherapy may become more cytotoxic. Double strand DNA break repair is mediated by either non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ repairs through direct ligation of a double stranded break, whereas HR utilizes a homologous template to recover the wild type sequence. Reporter cassettes involving the expression of green fluorescent protein were used to distinguish between these repair mechanisms. Titrations with etoposide show that a logarithmic increase in drug concentration yields a corresponding increase in repair through HR. This result demonstrates that topoisomerase II mediated damage is efficiently repaired by the process of HR. Additional experiments with another reporter cassette indicated that repair of topoisomerase II mediated DNA damage occurs more efficiently through the HR pathway than the NHEJ pathway. Collectively, the data suggest that tumor cells proficient in HR repair may effectively elude treatment by topoisomerase II targeting drugs.

KEYWORDS: topoisomerase, chemotherapy, DNA damage, homologous recombination, nonhomologous end joining

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INTRODUCTION

Topoisomerase poisons are widely used as anti-cancer chemotherapeutics [2]. Topoisomerase IIα (topoIIα) is the target for many anti-cancer agents because cancer cells have increased mitotic activity, requiring an increase in topoIIα expression.

Topoisomerase IIa Mechanism

TopoIIα stimulates relaxation, decatanation, and unwinding DNA during replication and cellular division (Figure 1). A prime example of topoIIα catalytic activity is during DNA replication when the replication fork melts the DNA hydrogen bonds between base pairs. As a result, the DNA preceding the replication fork begins to wind into a highly taut coil called a supercoil. If left alone, this negative supercoiling can be so severe as to fracture the DNA itself, thereby creating a genotoxic event [2]. TopoIIα relaxes the supercoiling.

TopoIIα is homodimer with a Mg2+ cation per dimer. TopoIIα exists as closed or open clamps dependent upon ATP binding. ATP binding switches topoIIα from an open to closed clamp formation. In each subunit of human topoIIα, the Mg2+ cation stabilizes the tyrosine – 804 residue, thus allowing a nucleophillic attack of the 5' phosphodiester bond [2]. The mechanism is repeated on both sides of the double helix. As a result, TopoIIα becomes covalently bound and creates a protein-DNA adduct with a double stranded break (DSB). Transient strand passage translocates the uncut strand through the DSB. Within the active site, the dissociated ends are religated. ATP hydrolysis then switches the homodimer to the open conformation. This mechanism is equilibrated and can either increase or decrease the linking number by two, meaning that both phosphodiester backbones are translocated, tightening or relaxing the helical structure. The isozyme topoIIβ is not mitotically stimulated and is poorly understood, but it is known to share this mechanism [2] (Figure 1).

Topoiomerase I Mechanism

Topoisomerase I (topoI) is relevant to mitotic, transcription, and promoter regulation [2]. TopoI does not require ATPase activity. A tyrosine residue performs a nucleophilic attack on the 5' phosphodiester bond, creating a single stranded gap. TopoI transfers the free 3' end about the intact strand and religates the gap within the catalytic site. The topoI mechanism is in equilibrium, allowing for the increase or decrease in linking number by one through the pivoting of one phosphodiester backbone around the other.

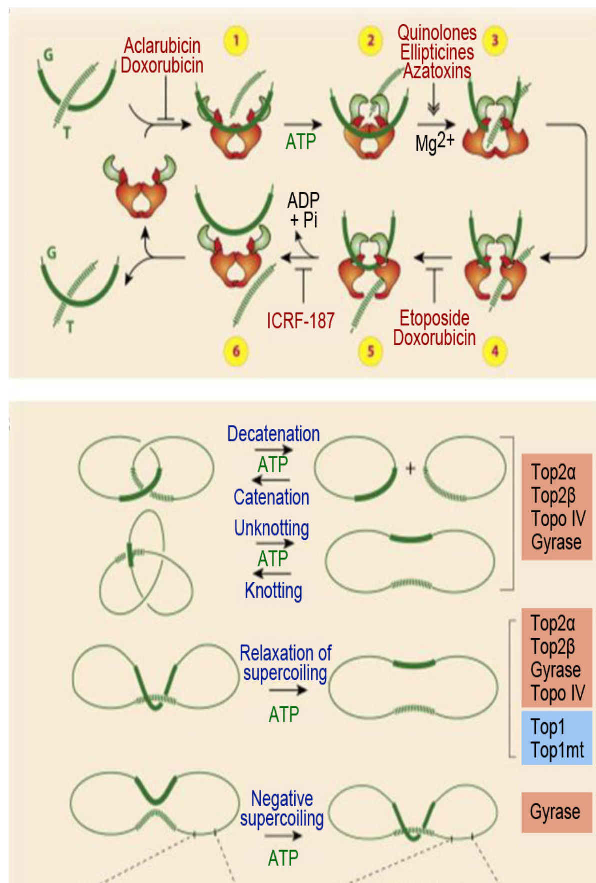

Figure 1 - A. Topoisomerase II Poison; B. Topoisomerase II Enzyme Mechanism [2]

Topoisomerase IIα Poisons

TopoII poisons such as etoposide (VP16) stabilize enzyme/DNA cleavages and fragment the genome (Figure 1). Many topoIIα agents are in clinical use and are approved by the Food and Drug Administration [5]. Therefore, it is vital that an understanding of how topoIIα breaks are repaired allowing cancer cells to elude treatment is necessary. VP16 itself is a widely used chemotherapeutic agent and readily available, thus will be the focus of this project [5].

Chemotherapy sometimes requires high dosages of topoIIα agent to ensure that DNA damage does not undergo repair, as the cleavage complex is a transient and

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reversible event [2]. The stabilized DSB created by topoIIα poisons increase the half-life of the cleavage complex. DNA/topoII complexes are processed by the 26S proteosome, a macromolecular structure that degrades ubiquitinated proteins, thus removing the topoIIα polypeptide portion and leaving a DSB (Figure 2) [4]. Recent studies indicate that the removal of the topoIIα protein can be performed through CtIP and the phosphodiesterases TDP1 and TDP2 [9]. If the DNA damage is not efficiently repaired, the cell will undergo apoptosis. This could possibly reduce the amount of agent needed to fight the malignancy. Information on the repair process can lead to new strategies that can inhibit the reversal of topoIIα mediated DNA damage, thereby minimizing patient side effects through the increase of drug efficacy.

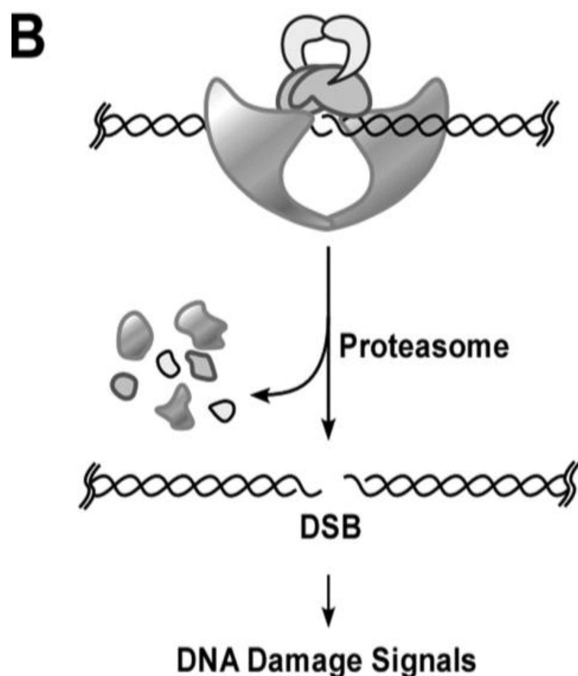

Figure 2 - TopoII Cleavage Complex Repair [4].

DSB Repair Pathways

DSBs are common events. The dissociated ends created by DSBs can reassociate indiscriminately, differing in sequence from the wild type and thereby creating chromosomal translocations [7]. To circumvent this, cells evolved two known mechanisms to correct the DSB: non-homologous end joining (NHEJ) and homologous recombination (HR).

NHEJ is the main pathway by which healthy cells repair DSBs; however, this can alter gene regulation or expression (Figure 3). The process involves the direct ligation of a DSB without regard to sequence homology or phase of the cell cycle [12]. NHEJ is a low fidelity, high mutation prone pathway, but repairs DSBs rapidly [12]. Ku, a heterodimer of Ku70 and Ku80, recognizes the DSB and initiates the NHEJ repair pathway [12]. The Ku protein attracts DNA-PKcs by forming a holoenzyme and autophosphorylates itself, possibly providing the energy needed for the subsequent blunt ligation. NHEJ provides genomic stability with a half life of 30 minutes [12].

HR is a high fidelity but time-consuming pathway occurring mainly in the late phases of the cell cycle [1]. HR commences upon DNA damage recognition, and a cascade of signaling recruits proteins that further resect the break to single stranded 3' ends (Figure 3) [8]. The single stranded ends are then coated with single stranded binding proteins, protecting the templates. These unbound ends are then wrapped with Rad51, which is associated with BRCA1. With Rad51 bound, the single stranded DNA participates in homology recognition [1]. The Rad51 complex also allows for strand exchange. Subsequent branch migration and nucleotide polymerization from DNA polymerase II occur. The whole complex then resolves itself with an exact copy of the template homolog where the DSB occurred. Most non-cancer cells are in the resting phases of the cell cycle and thus are not subjected to the HR pathway often.

Goal

This research project analyzes whether topoII/DNA damage complexes are repaired through either HR or NHEJ. DNA repair events resulting from poison damage can be quantified through the use of a highly specific reporter cassette for either HR or NHEJ. With this experimental system, we found that HR is the preferred DSB repair pathway in HeLa cells. This knowledge could lead to increased efficacy of anti-cancer chemotherapeutics by blocking HR pathway proteins and/or signaling.

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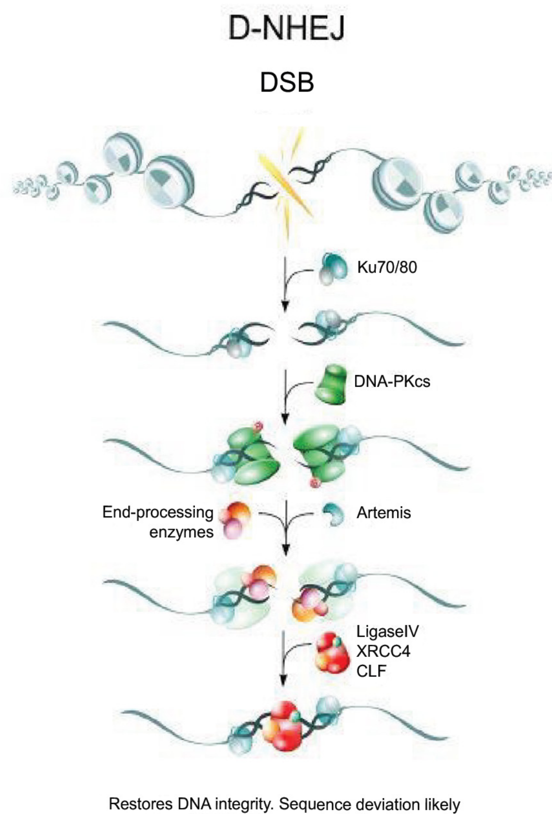

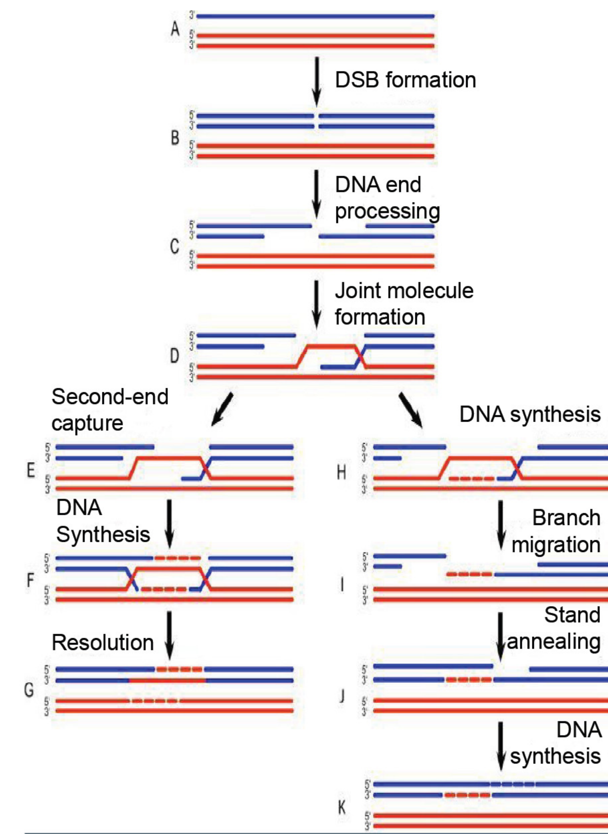

Figure 3 - NHEJ [11] and HR [7] Pathways. NHEJ uses the Ku complex to recruit subsequent proteins such as DNA-PKcs, ultimately resulting in blunt double strand ligation of the DSB. HR is a high fidelity pathway that uses a donor sequence as a template, thus resulting in high fidelity DNA retrieval.

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METHODS AND MATERIALS

NHEJ and HR Reporter Plasmids

The reporter plasmids for both NHEJ and HR employ a similar approach. The presence of the restriction endonuclease ISce-I produces a highly specific DSB at the designated ISce-I cut site (Figure 4). NHEJ or HR can then be used to repair the DSB. With HR, the second cassette has a homologous sequence that allows repair of the DSB, expression of GFP. The NHEJ reporter plasmid has the ISce-I cut site centrally located between the GFP sequence, with GFP expression upon blunt ligation.

Exposure of HR HeLa cells to Etoposide and ISce-I

HR HeLa cells were transfected with pISce-I using Lipofectamine Reagent 2000 (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. At 24 hours post transfection, the cells were treated with the desired concentration of VP16 for one hour. Cells were then incubated for 24 and 72 hours in the absence of VP16 to establish a time course for recovery. At the end of each respective recovery incubation time, GFP fluorescence was read using FACSCalibur Fluorescence Activated Cell Sorter (FACS) and CellQuest software (BD Bioscience, San Jose, CA).

Doxycycline inducible ISce-I expression system

The inducible reporter system used for the NHEJ and HR HeLa cells lines (Figure 4) were stably integrated by Dr. Bongyong Lee. The RY-HR HeLa cell line is not doxycycline inducible. These reporters use a tetracycline induction system to transcribe ISce-I endonuclease. Doxycycline is a semi-synthetic tetracycline derivative. Following exposure to doxycycline, the pCMV promoter sequence is exposed, allowing for transcription of the ISce-I downstream gene. Without doxycycline exposure, no ISce-I is expressed. Expression of the ISce-I restriction enzyme creates a DSB at the ISce-I cut site (Figure 4), thereby allowing the cells to perform DSB repair.

Analysis of Repair of Topoisomerase I and II DNA Damage by HR and NHEJ Pathways

Stably integrated RY-HR HeLa cells and inducible, stably integrated NHEJ HeLa cells were observed to be in exponential growth. The plates were then exposed to an increase in VP16 concentration, and an increase in campothecin (CPT), a topoisomerase I poison, for one. A positive control and a transfection efficiency control were set up by the transfection of pISce-I and pEGFP utilizing Lipofectamine 2000 reagent, according to manufacturer's instructions. Single plates for both lines were left untreated and untransfected as a negative control. The plates were then incubated for three days following the drug treatments. After incubation, the supernatant of the plates and the trypsinized cells were transferred to a 15 mL conical tube. The cells were analyzed via FACSCalibur FACS and CellQuest Pro (BD Software) for GFP expression.

Screening of RYHR-GFP clones

HeLa cells stably integrated with the RYHR plasmid (TopoGEN, Daytona Beach, Florida) were provided by Alex Fagenson (Muller lab, UCF). Colonies were selected as single clones and were subcultured to expand the clones. Clone screening was performed by transfecting pISce-I with Lipofectamine Reagent 2000 for five hours. The cells were then incubated in the absence of pISce-I for 24, 72, and 144 hours to establish a time course for recovery. Using the FACSCalibur FACS and CellQuest software, GFP expression was calculated. The best clone was kept for further projects, and the remaining clones were discarded.

Analysis of HR in RY-HR Stably Integrated HeLa Cells Using VP16

Making sure the cells were in heavy exponential growth, increasing VP16 concentrations were added and exposed continuously for five days until 100% confluency. In separate subcultures, a titration of VP16 was exposed to the cells for one hour. One plate was left untreated, and the last plate was exposed to five µg of ISce-I for four hours with Lipofectamine Reagent 2000 according to the manufacturer's instructions in 5 mL OPTI-MEM I. The cells were then grown to confluency without any additives. Using the FACSCalibur and CellQuest software, GFP expression was read.

Trypan Blue Exclusion Assays for Cell Viability

RY-HR HeLa cells and NHEJ HeLa cells were treated with increasing VP16 concentrations. The cells were exposed to VP16 for one hour. After three days incubation without VP16, trypan blue dye was aliquot to microcentrifuge tube. The supernatant of each well was saved

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in a 15 mL conical tube. The cells were trypsinized and added to the supernatant. From the cell suspension, 20 μL was added to the 180 μL of 0.1% trypan blue dye. From that, 10 μL of the dyed cells were injected into a hemocytometer slide and viewed under a microscope. The slide was divided into four quadrants, and each of the four quadrants was counted and totaled. A ratio of stained versus unstained cells indicates percentage cytotoxicity.

Confocal Microscopy

The three lines of HeLa cells were seeded on a cover slip. ISce-I was transfected using Lipofectamine Reagent 2000 for the non-inducible RYHR clone was added, according to manufacturer's instructions, for four hours, and doxycycline was added to the media for the inducible systems. One hour drug treatments of VP16 were then performed at varying concentrations. Following drug treatment, the cells were incubated for 24 and 48 hours without pISce-I or doxycycline exposure to establish a time course. Using a bent hypodermic needle, the cover slips were carefully removed. A 1.5 μM PI staining solution stained the cells for thirty minutes in the dark. Using FischerFinest Premium Slides, one drop of emulsion oil was placed between the slide and the cover slip. Clear nail polish was then used to seal the cover slip to the slide. Following drying, the slides were analyzed using a confocal microscope at 20X and 100X power.

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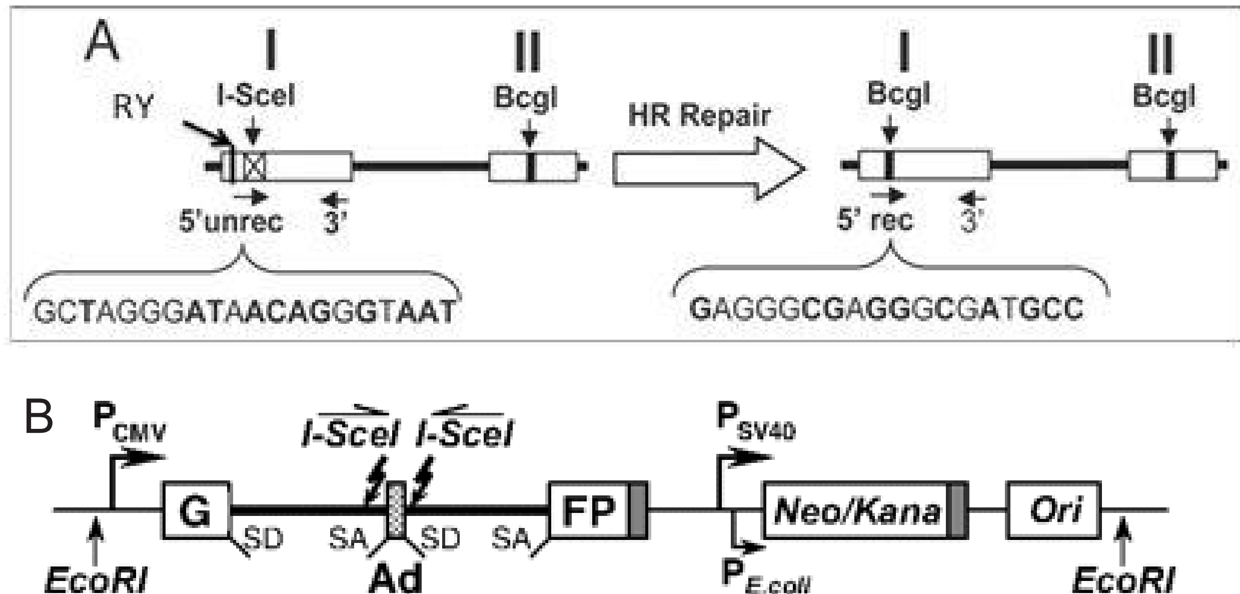

Figure 4 – The RY-HR and NHEJ Reporter Constructs.

(A): RY-HR reporter system. The RY element and the ISce-I loci create DSBs when exposed to a topoIIα poison or ISce-I restriction endonuclease respectively. Following a DSB, cassette I searches for homology amongst cassette II, leading to HR repair of the DSB and GFP expression. Before HR repair, cassettes 1 and II are incapable of expressing GFP due to mutations in the first cassette and a stop codon in the second cassette. Following HR repair as indicated by the diagram, cassette I can express GFP.

(B): The NHEJ reporter system. The opposing ISce-I restriction cut sites allow for direct ligation of the opposing cassettes initiating GFP expression. The RY sequence is not needed due to the size of the NHEJ reporter system. The RY-HR reporter cassette is substantially smaller than the NHEJ reporter cassette, thus a targeting agent is needed to initiate HR using a topoIIα poison.

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RESULTS

HR analysis following VP16 drug treatments and transfection of ISce-I in HR HeLa cells

The treatment of HR HeLa cells with VP16 served as an initial and essential founding piece of information for the following results (Figure 5). The positive control, which includes the transfection of ISce-I with no subsequent drug treatment, illustrates that the reporter system is working and shows the potential of GFP expression. The negative control consists of HR cells, lacking ISce-I transfection and drug treatment. These cells provide a background reading of fluorescence that is ultimately subtracted from the corresponding ISce-I data points. Although the anticipated GFP expression for the negative control should be 0%, the 0.1% GFP finding is likely due to the nature of the integrated GFP cassettes or autofluorescence. An autofluorescence control was not obtained.

The combination of VP16 with the transfection of ISce-I increased the GFP expression at 72 hours by 1.3% in the 5 μM VP16 range. GFP expression at 72 hours steadily declined with VP16 concentrations higher than 5 μM (Figure 5). In addition, GFP expression gradually decreased at 24 and 48 hours with VP16 concentrations above 5 μM. This is likely due to toxicity of the VP16 drug (IC50 = 200 μM). GFP fluorescence in general is low due to the nature of exposing a poison to cells; the surviving, fluorescing cells are most likely survivors of the poison exposure.

Due to the low percentages and small size of the HR reporter cassette, a topoIIα targeting sequence was used. The RY-HR (Figure 4) cassette contains an ultra high affinity 56 base pair purine rich sequence for topoIIα[10]. This high affinity topoII binding site, the RY site, should attract the endogenous topoII and direct cleavages in this region 5' of the ISce-I site in the presence of a drug such as VP16. As a result, the cellular DNA damage repair system should be activated with the HR cassette. Two exposure times of VP16 were allotted for the RY-HR HeLa cells. One batch of cells was exposed to VP16 for five days. The second batch was exposed to VP16 for one hour and then allowed to recover for five days. The positive control is solely a transfection of ISce-I plasmid while the negative control represents background GFP expression in the absence of HR induction through ISce-I transfection or drug treatment. The results show that HeLa cells are able to repair the VP16 initiated DSB up to 5 μM concentration (Figure 6). There is a clear trend towards increasing GFP expression with increasing concentration of VP16 treatment in both the prolonged and short drug exposure time frames. During the five day exposure, there is an increase of GFP beyond the positive control. The one hour drug treatments also evinced a trend of increasing GFP expression with increased dosage of VP16. The data suggest that the presence of the RY element, the ultra high affinity topoIIα binding site, is stimulating HR in the GFP reporter through VP16 drug treatment. In this analysis, cells were treated at low to high levels of VP16 for a total of five days or with the same concentration for one hour followed by five days of recovery. In both cases, it is clear that VP16 is inducing expression of GFP through HR at drug concentrations (0.05 – 50 μM). This indicated that the repair of topoIIα induced DNA damage can occur through HR. Higher concentrations of VP16 (> 50 μM) were toxic to the cells. As a result of this, we decided that a morphological investigation of the GFP expressed in HR, RY-HR, and NHEJ HeLa cells was required.

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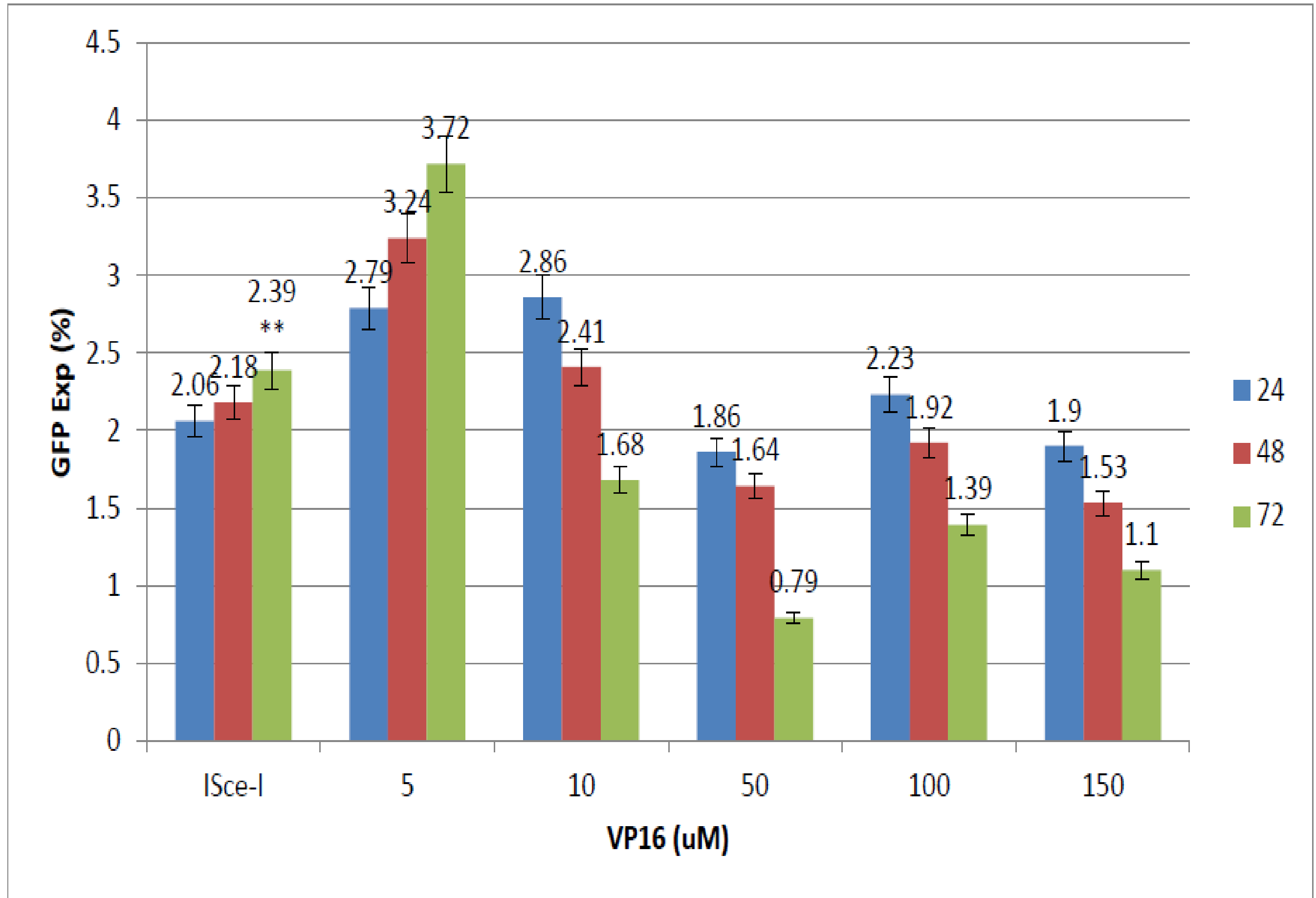

Figure 5 - HR in VP16 Treated HR HeLa Cells. HR HeLa cells were transfected with ISce-I and treated with VP16 for 1 hour. A time course for recovery for 72 hours (p<0.01) was read for GFP expression using FACS analysis. GFP expression is indicative of a HR event. Standard error bars symbolize data range after three replicated trials.**

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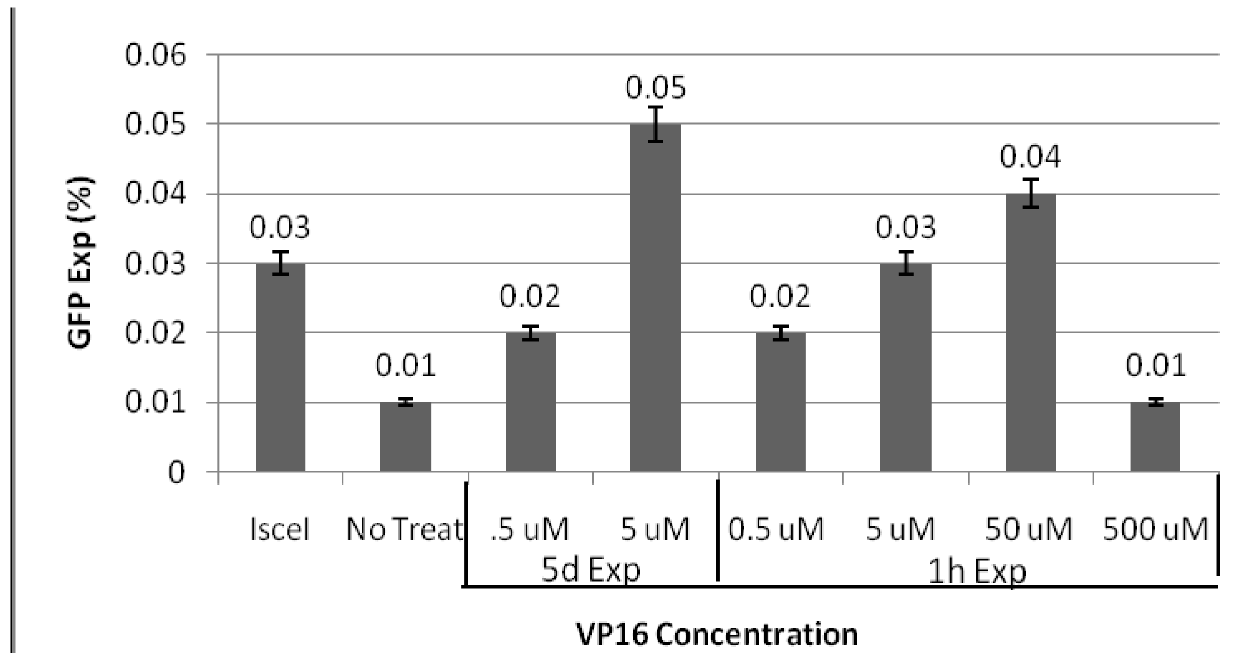

Figure 6 - HR in VP16 Treated RY-HR HeLa Cells. RY-HR HeLa cells were treated with VP16 for the indicated time frame (5d = 5 days, 1h = 1 hour) at the stated VP16 concentrations. One hour drug treated cells were incubated for the remaining 5 days in the absence of VP16. All cells were harvested simultaneously for GFP analysis via FACS (**p<0.4). Standard error bars symbolize data range after three replicated trials.

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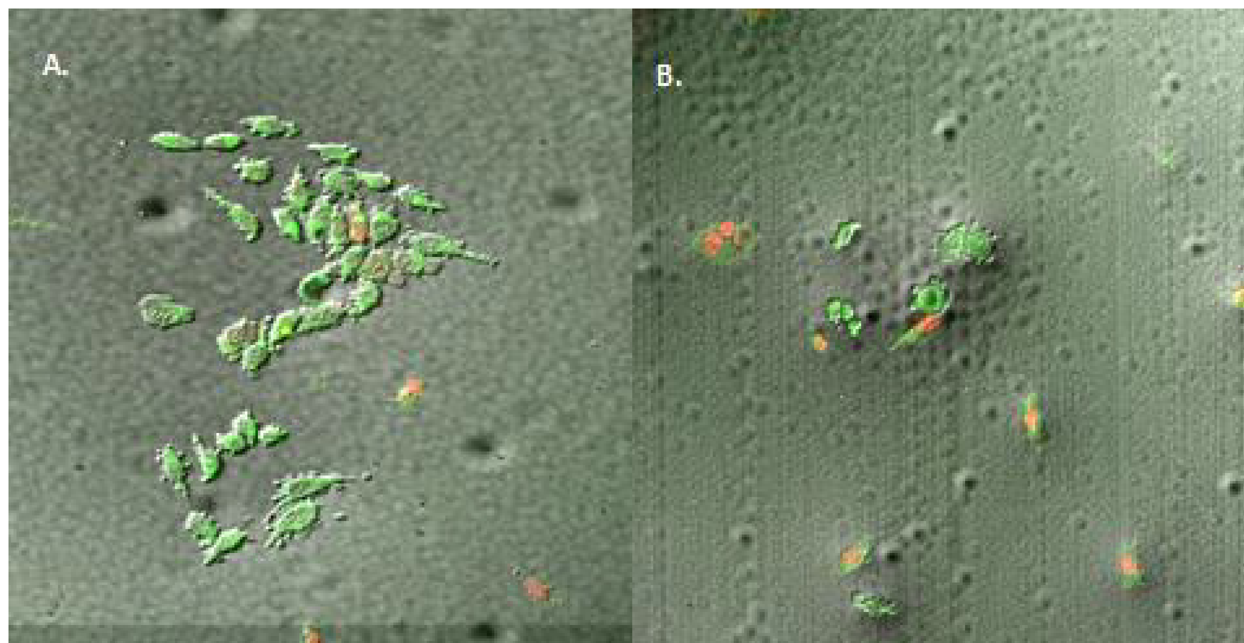

Figure 7 - Confocal Microscopy of RY-HR HeLa Cells. RY-HR HeLa cells were seeded over a cover slip and incubated for 24 hours. Following incubation, 5 μM VP16 (A) and 100 μM VP16 (B) were exposed to the cells for one hour. The cells were then incubated for two days without VP16 exposure. The cover slips were then removed and exposed to a PI staining solution for 30 minutes. Confocal microscopy was viewed with fluorescence for GFP and PI. PI stains the nucleus red and the GFP fluoresces green. GFP expression is indicative of a HR event. (A) and (B) were captured at 20X magnification.

Confocal Microscopy

Confocal microscopy was performed to examine GFP expressing cells morphologically (Figure 7 – 8). The GFP protein appears to be well distributed throughout the cell in the RY-HR cultures treated with VP16 for one hour followed by a 48 hour recovery interval (Figure 7). At 100 μM VP16, there is an obvious cytotoxic effect on the cells using microscopic observations. Confocal analyses were performed on the doxycycline inducible NHEJ and HR reporter cells, to examine any cytological differences between these two reporter systems. The NHEJ reporter yielded cells with GFP distributed throughout the cell (Figure 8). In contrast, the GFP produced as a result of HR in the HR HeLa cells without the RY element appears largely in the nucleus, possibly due to a nuclear localization sequence on the GFP. GFP is indicative of a HR or NHEJ event; cells that do not express GFP have not expressed either of these pathways.

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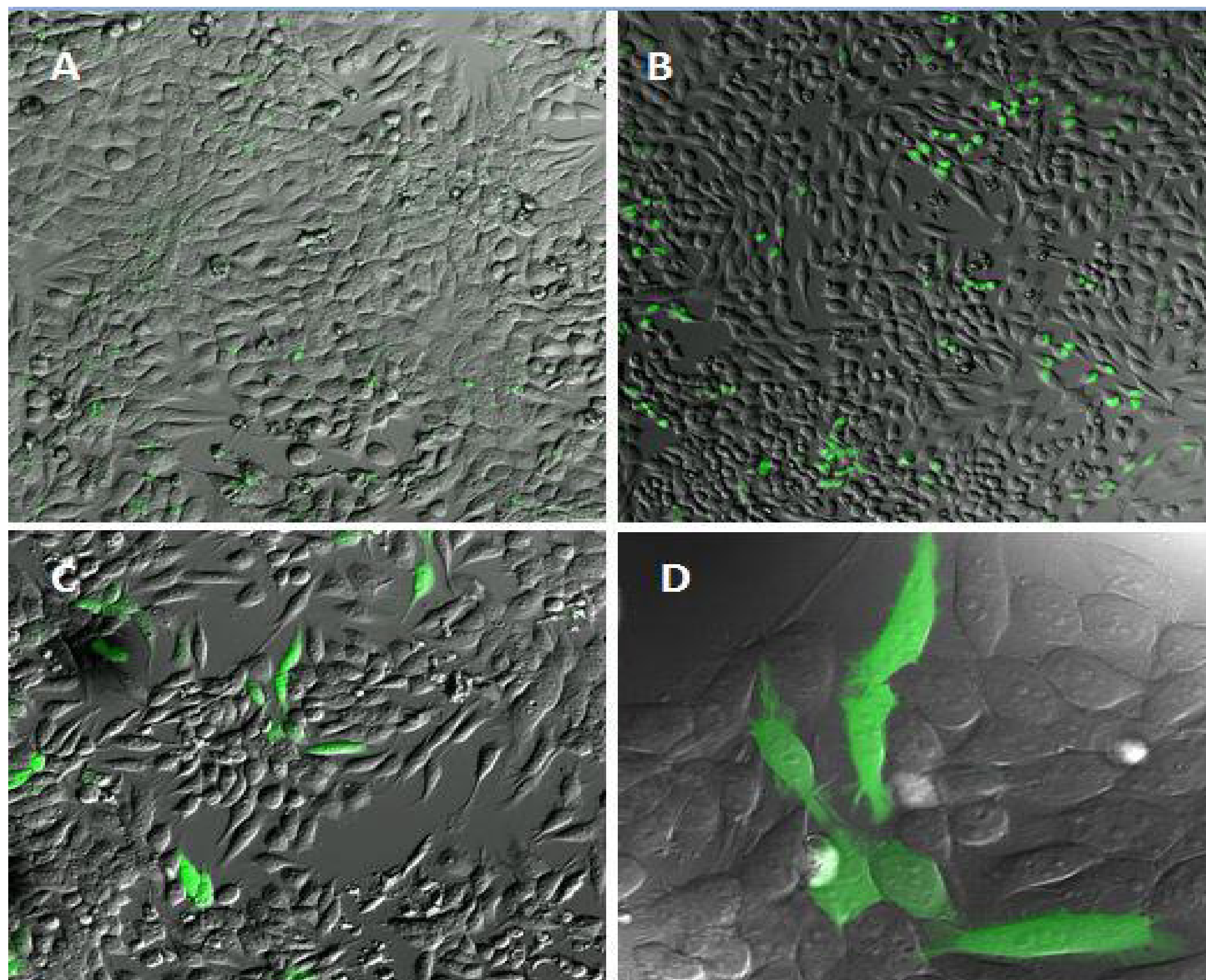

Figure 8 - Confocal Microscopy of HR and NHEJ HeLa Cells. Doxycycline inducible NHEJ and HR HeLa cell lines were seeded with an inherent slide base. Following one day of incubation, doxycycline was added to the stated wells. The cells were incubated with the doxycycline for three days. (A) Shows the uninduced HR HeLa cells at 20X zoom. (B) Shows doxycycline induced HR HeLa cells at 20X zoom. (C) Shows doxycycline induced NHEJ reporter system integrated HeLa cells at 20X zoom. (D) shows cells from (C) at 100X zoom. The confocal microscope was set to excite solely GFP. GFP expression coincides with the NHEJ or the HR repair pathway utilization.

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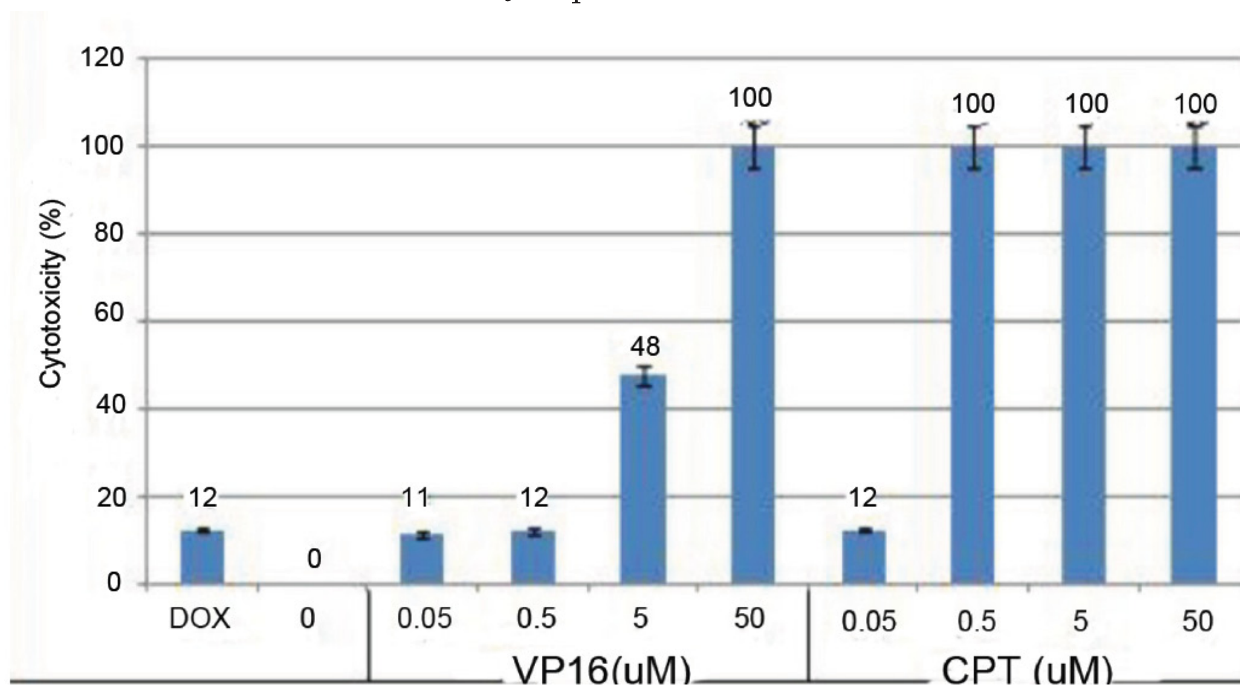
NHEJ Reporter HeLa cells



RY - HR Reporter HeLa Cells

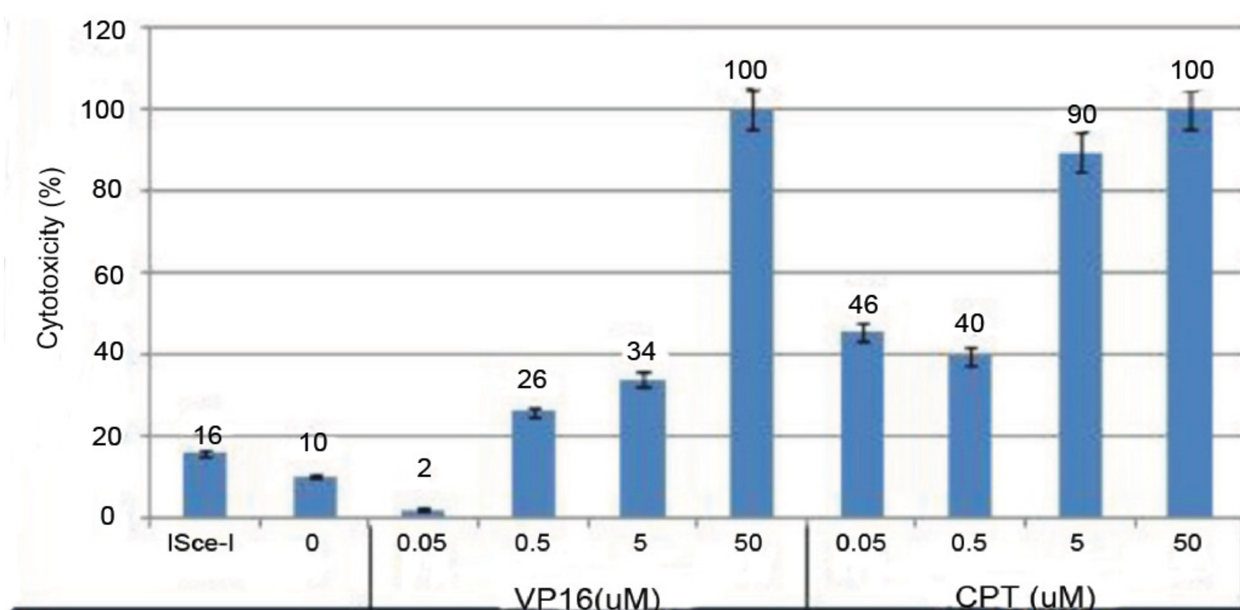


Figure 9 - Cytotoxicity for NHEJ and RY-HR HeLa Cells. Cytotoxicity percentages were calculated for NHEJ reporter in HeLa cells and RY-HR HeLa cells. Drug treatments lasted for one hour with both VP16 and CPT (TopoI Poison). The cells were then washed and allotted a recovery interval of 48 hours. Cytotoxicity percentages were calculated using trypan blue exclusion assays.

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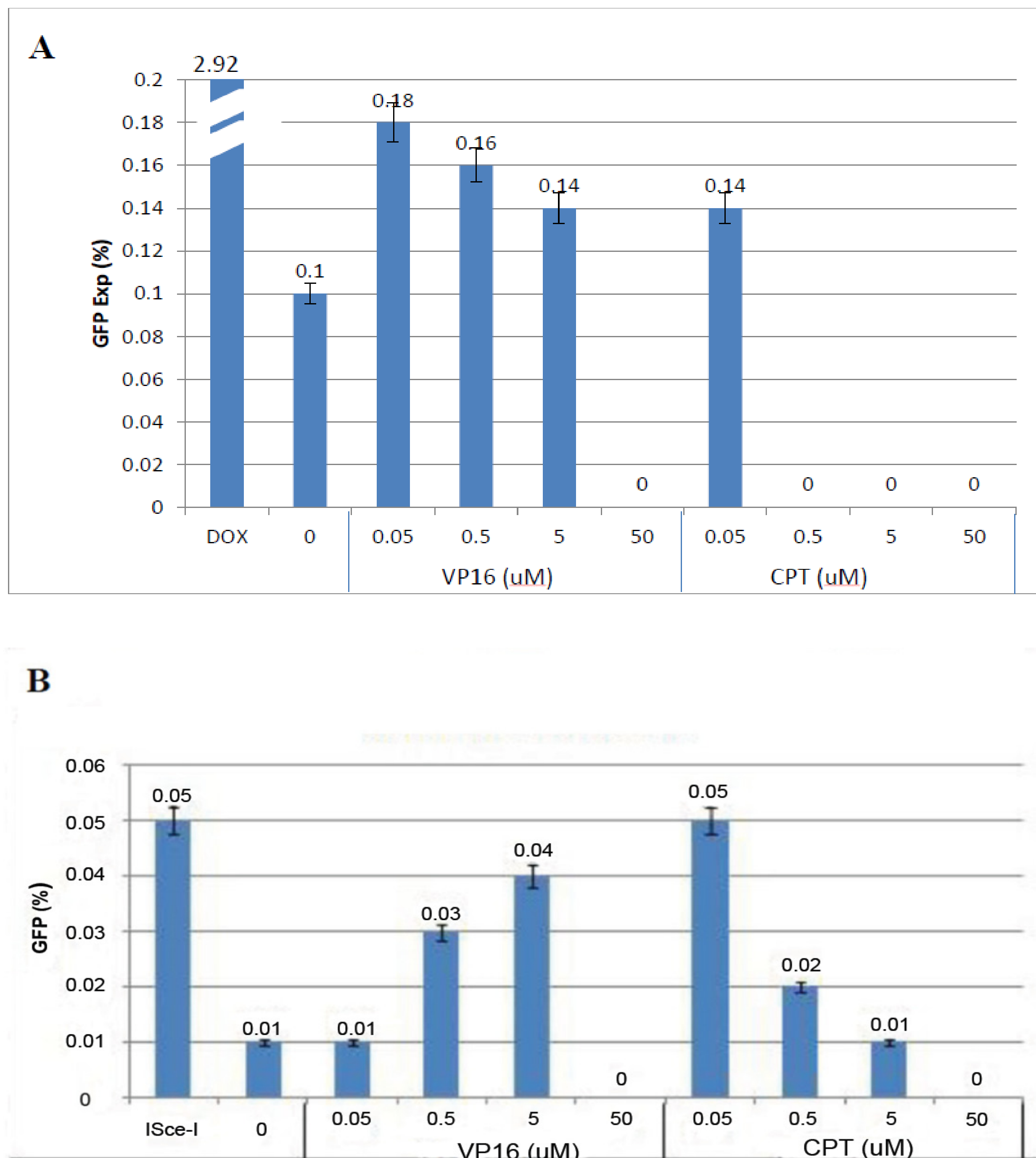

Figure 10 - HR and NHEJ after VP16 Treatments (A). Illustrates GFP expression from NHEJ reporter cassettes in HeLa cells. (B) Shows GFP expression from RY-HR HeLa cells. Drug treatments lasted for one hour for both VP16 and CPT (TopoI poison). For the positive controls: ISce-I transfection lasted for four hours and three day exposure for the DOX inducible NHEJ system. The drug treated cells recovered for three days and were analyzed for GFP expression using FACS. Transfection efficiency was measured by pEGFP (18%).

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Analysis of Toxicity

The results of the trypan blue exclusion assay shows that with increasing concentration of VP16, there is an increase in cytotoxicity as expected (Figure 9). VP16 is a known chemotherapeutic and thus should have a high toxicity rating as shown in these results. CPT, a highly specific TopoI poison, shows an increased cytotoxicity amongst the NHEJ reporter system with concentrations from 0.5 – 50 μM being toxic. The RY-HR HeLa cells show increased resistance to CPT. The RY-HR toxicity data show that VP16 is generally less cytotoxic than CPT with regard to HR, which indicates that HeLa cells may be able to repair more efficiently the damage caused by a topoIIα poison.

Analysis of Repair of Topoisomerase I and II DNA Damage by HR and NHEJ Pathways

There are two major DSB repair pathways in animal cells: HR and NHEJ. Since we have dedicated reporters for each pathway and given that the drugs are highly specific for topoI (CPT) or topoII (VP16) mediated DNA damage, the repair process was evaluated in each case (Figure 10). In figure 10-A, the NHEJ reporter system shows there is an increase in susceptibility in CPT damage and mild reparability in exposure to VP16 with dose dependent decrease in GFP. The positive control is noticeably elevated in comparison to the remaining samples. There is a decrease in NHEJ repair with an increase in VP16 concentration. Figure 10-B shows the effects of CPT and VP16 on RY-HR HeLa cells. There is an increase in HR with an increase in VP16 concentration and the opposite trend with CPT concentration. GFP expression percentage remains low in the RY-HR HeLa cells due to the cytotoxicity of the introduced poisons, and also the expression abilities of the clones as noted by the positive control. In both instances, 50 μM of CPT or VP16 caused toxicity in the culture (see also Figure 9). The data points show that VP16 and CPT HR repair gives a GFP reading close to that of the positive control, ISce-I transfection. These trends may mean that HR is the preferred pathway utilized by HeLa cells to repair from topoIIα and topoI poison damage.

DISCUSSION

This project utilized reporter cassettes to analyze the repair of DNA damage induced by topoIIαand topoI poisons. Specific cassettes were employed that report repair of DSBs by either HR or NHEJ (Figure 4). The first cassette, in the case of RY-HR cultures, contains the topoIIα hot spot for the topoIIαpoison and/or an ISce-I restriction endonuclease cut site. The second cassette contains the template sequence to repair the induced DSB in the first cassette. Without drug treatment or pISce-I transfection, GFP is transcribed but not expressed due to mutations in the first sequence and a stop codon in the second sequence (Figure 4). The NHEJ pathway was specifically measured using the NHEJ reporter (Figure 4). With the NHEJ reporter, the formation of wild type GFP cannot proceed by HR due to the lack of a homologous donor sequence. The expression of GFP from the NHEJ reporter cassette can thus only result from a NHEJ event.

The RY-HR GFP reporter systems indicated that, with an increase in VP16 concentrations to 5 μM (Figure 10 - B), there is an increase in HR repair. A dose dependent decrease in the NHEJ pathway (Figure 10 – A) in response to VP16 was also observed. The RY-HR integrated cells showed a dose dependent decrease in HR treated with the topoI poison, CPT (Figure 10-B). The DSB initiated by VP16 appears to be repaired through the high fidelity, error free HR pathway. The high mitotic activity of cancer cells could make the cell cycle dependent HR repair pathway preferential compared to the non-cell cycle dependent NHEJ pathway. Most healthy cells are in the resting G1/G0 phase and, thus are not prone to HR DSB repair.

The data in Figure 9 define the toxicity profile of VP16. The highly specific topoI poison CPT had a higher toxicity than the VP16. HR may well be the preferred DSB repair pathway because the cancer cells are constantly dividing and HR is cell cycle specific. Moreover, HR proteins are associated with specific cell cycle checkpoints, whereas NHEJ is not [14]. Due to the high mitotic levels of most cancer cells, HR should be a prominent pathway for break repair. However, not all cancer cell types follow the HR pathway preferentially. For example, both normal and malignant urothelial cells upregulate the NHEJ pathway, while HR is more dominant in cervical cancer cells [15]. The data and literature suggest that DSB repair pathways are highly variable and may well be dependent on tumor tissue

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location. Most importantly, since healthy cells preferentially use NHEJ to repair DSBs, a selective agent for HR proteins could sensitize cells to anti-cancer treatments while leaving healthy cells relatively unharmed. One report demonstrated that NHEJ targeting sensitized tumor cells and caused normal cell damage [16]. The targeting of HR could lessen the malevolent impact of chemotherapy on healthy cells, and thereby target cancer cells selectively.

These data may illustrate that HeLa cells utilize the HR pathway over the NHEJ pathway to repair from the exposure of VP16, a topoIIα poison. Future work for this project includes analyzing, through the same reporter constructs, the effects of VP16 and CPT drug treatments on cell lines known for specific repair pathway upregulation. In addition, topoIIβ analysis can be performed based on the two pathways. TopoIIβ has been found to be a causative enzyme of secondary malignancies in cancer patients treated with topoII poisons. Finally, knockout or over expression of specific proteins upregulated in HR such as BRCA1 can be explored for its effects on both pathways via the GFP system.

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