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MUTAGENIC AND TUMOR SUPPRESSOR FUNCTIONS OF DNA POLYMERASE IOTA IN MAMMALIAN CELLS

By

Lindsey Jay Stallons
B.S., UofL, 2006

A Thesis
Submitted to the Faculty of the
Graduate School of the University of Louisville
in Partial Fulfillment of the Requirements
for the Degree of

Master of Science

Department of Pharmacology and Toxicology
University of Louisville
Louisville, KY

August 2008

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A Thesis Approved on

April 28, 2008

By the following Thesis Committee:

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Thesis Director

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DEDICATION

This thesis is dedicated to my wife
Mrs. Stacey Elaine Stallons
who stands by me and supports me while I follow my dreams.

ACKNOWLEDGMENTS

I would like to thank my mentor, Dr. W. Glenn McGregor, for his guidance and support. I would also like to thank my other committee members, Drs. Jason Chesney, Ramesh Gupta, Christopher States, Brian Wattenberg, and Wolfgang Zacharias, for their insight into my project. Finally, I would like to thank Tom Burke for passing on his technical knowledge and contributing to the experiments described here.

ABSTRACT

MUTAGENIC AND TUMOR SUPPRESSOR FUNCTIONS OF DNA POLYMERASE IOTA IN MAMMALIAN CELLS

Lindsey Jay Stallons

August 7, 2008

The Y family of DNA polymerases in higher eukaryotes contains at least four members which are implicated in potentially error-prone replication through unrepaired damage in the genome. These proteins are encoded by the *REV1, POLH, POLI, and POLK* genes. An inherited deficiency in one of these DNA polymerases (POLη) is the molecular defect in the cancer prone xeroderma pigmentosum (XP) variant syndrome, making POLη the most studied member of this family. However, there exist critical gaps in our knowledge on the function of the other known Y family members (POL ι, POL κ, and REV1). The goal of this proposal is to investigate the in vivo function of DNA POLι, and the hypothesis that DNA polymerase ι acts as a mutagenic polymerase in translesion synthesis and as a tumor suppressor through a separate mechanism. To test this hypothesis, the mutagenic effects of chemical carcinogens which form structually different adducts will be examined in cells lacking POLη and/or POLι. To characterize the tumor suppressor function(s) of POLι, cell cycle progression will be monitored after UV in POLι null cells along with global gene expression. Finally, a novel mouse model will be used to determine the effect of polη and/or poli deficiency on UV- and chemically-induced skin cancer.

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CHAPTER 1

INTRODUCTION

The Y family of DNA polymerases in higher eukaryotes contains at least four members that are implicated in potentially error-prone replication through unrepaired damage in the genome. These proteins are encoded by the *REV1, POLη, POLι*, and *POLκ* genes. An inherited deficiency in one of these DNA polymerases (POLη) is the molecular defect in the cancer prone xeroderma pigmentosum (XP) variant syndrome, making POLη the most studied member of this family. However, there exist critical gaps in our knowledge on the function of the other known Y family members (POLι, POLκ, and REV1). Our goal is to investigate the cellular function of DNA POLι, and we hypothesize that DNA polymerase ι acts as a mutagenic polymerase in translesion synthesis and as a tumor suppressor through a separate mechanism. This hypothesis is based on the following observations. First, *in vitro* studies with purified POLι have characterized its error-prone bypass of damaged DNA templates. Second, our group and others have shown that POLι is active in error-prone bypass of UV damage *in vivo*. Third, *polη*$^{-/-}$ *poli*$^{-/-}$ double knockout mice develop tumors faster than *polη*$^{-/-}$ *poli*$^{+/+}$ single knockouts despite having lower UV-induced mutation frequencies in their fibroblasts. Based on these observations, the focus of this thesis is on the cellular function of DNA POLι.

In Chapter 2, we will evaluate the mutagenic properties of chemical carcinogens in mammalian fibroblasts deficient for Y-family polymerases. We will examine the differential cytotoxic and mutagenic response of human and mouse fibroblasts with

decreased expression of POLη and/or POLι to benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), and N-acetoxy-2-acetylaminofluorene (AAAF). These chemicals form adducts at different positions in the DNA, and this Chapter is proposed to determine the cellular participation of each polymerase in bypassing chemically and structurally different DNA lesions.

Chapter 3 is designed to investigate the tumor suppressor role of murine polι. We propose to test the hypothesis that polι functions as a tumor suppressor in mouse cells. Our group showed that mice that are deficient in both *polη* and *polι* have lower UV-induced mutation frequencies, but strikingly develop tumors *faster* than mice that are deficient in *polη* alone. This unexpected result is contrary to the somatic mutation hypothesis of cancer and implies that polι has tumor suppressor activity that may be independent of its mutagenic TLS activity. To investigate this, we propose to examine the effect of polι deficiency on miRNA, gene expression, and cell cycle checkpoints induced by UV or genotoxic carcinogens.

Finally, we will characterize the effect of *polι* deficiency on *in vivo* skin tumorigenesis using a novel mouse model in Chapter 4. We hypothesize that deficiency in *polι* alone is sufficient to promote progression of skin cancer caused by UV and benzo[a]pyrene (B[a]P). Previous studies using C57BL/6J mice required UV treatment for 20 weeks, after which neither wild type nor *polι*$^{-/-}$ animals developed tumors. Our novel mouse model introduces combinatorial *polη* and *polι* knockouts into an SKH1/hairless *xpa*$^{-/-}$ background. These mice are nucleotide excision repair (NER)-deficient and develop tumors after only 8 weeks of UV irradiation. Using established models of UV- and chemically-induced skin cancer, we will be able to identify any previously unobserved effects of *polι* deficiency alone and in combination with *polη* deficiency during skin tumorigenesis. We will characterize the DNA damage and apoptotic response of epidermal cells in order to identify changes in these characteristics

due to proposed tumor suppression by polι. Finally, we will directly test the somatic mutation hypothesis of cancer by comparing *in vitro* mutagenesis results from Chapter 2 with *in vivo* carcinogenesis results.

Mutation and Cancer

Mutagenesis is a recognized cornerstone of every form of human cancer[1;28] dating back to the discovery of aneuploidy in cancer cells[12]. According to the modern somatic mutation hypothesis of cancer, a cell must undergo several independent genetic and epigenetic events that together confer properties associated with the malignant phenotype. These include acquisition of infinite lifespan, resistance to apoptotic signals, growth factor independence, resistance to antigrowth signals, angiogenesis, and tissue invasion[32]. Acknowledgment of the critical role of mutations in carcinogenesis opens the possibility of chemoprevention using antimutator strategies. Although recent advances have elucidated key details of the pathways underlying mutagenesis, there exist critical gaps in our knowledge of the fundamental mechanisms by which DNA is mutated during carcinogenesis. In this thesis, we will investigate how environmental carcinogens permanently alter the sequence of DNA in order to cause cancer.

Translesion Synthesis in Eukaryotes

Recent evidence implicates DNA polymerase episilon (ε) in leading strand DNA replication, and delta (δ) in lagging strand[65]. When these enzymes encounter adducts that distort the helix, a variety of bypass mechanisms are initiated. This is particularly true of adducts in the leading strand template. The signals that initiate these responses are poorly understood at the present time, and constitute active areas of investigation in many laboratories including our own. It has been shown using a plasmid containing a site-specific acetylaminofluorene adduct that error-free damage avoidance accounts for more than 92% of lesion bypass, and that mutagenic translesion synthesis accounts for only 0.3% of bypass events[4]. It is clear from these studies that error-free homologous

recombination is the preferred method to resolve blocked replication forks in *S. cerevisiae*, and experts in the field generally agree that these mechanisms are conserved in higher eukaryotic cells. Nevertheless, direct DNA synthesis past the lesion clearly occurs in all cells, and is the source of virtually all mutations induced by genotoxic carcinogens. This process is called translesion synthesis (TLS) and involves recruitment of Y-family DNA polymerases. The signaling cascade that initiates TLS is poorly understood. Data indicate that monoubiquitination of PCNA on K174 of each subunit is an early event that appears to promote interaction with REV1 and polη in yeast. This is also true of higher eukaryotic cells, but such cells have additional layers of complexity because they have two additional homologues (POLι and POLκ) that are not present in yeast. The cell biology associated with these enzymes, which presumably act in concert with REV1 and polη, is the subject of this thesis.

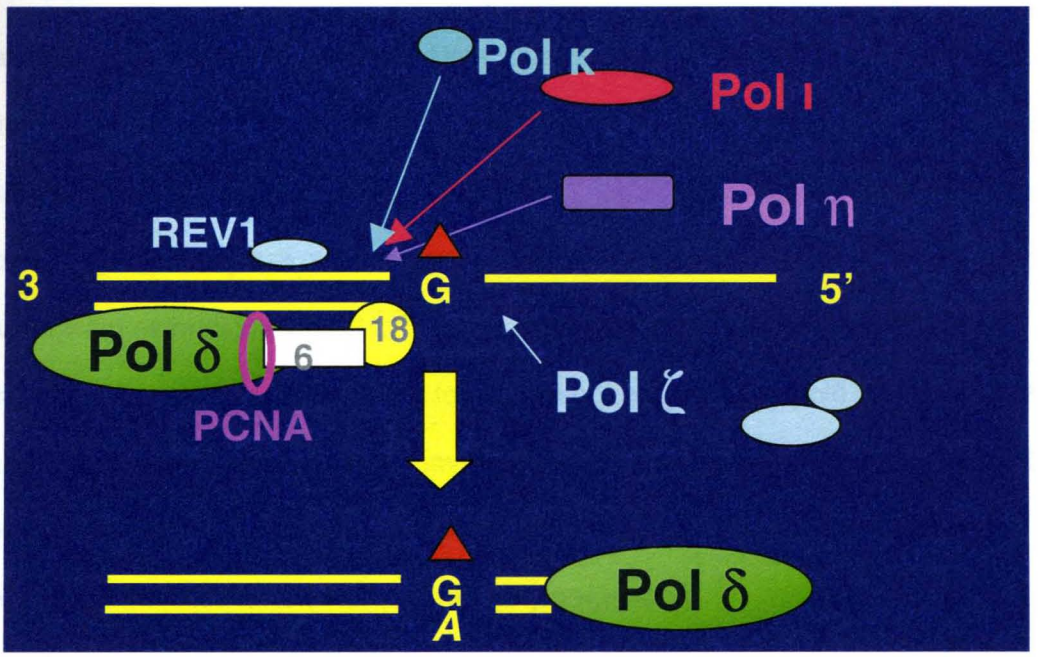

Figure 1.1. Model for translesion synthesis. DNA damage causes arrest of the replication fork and recruitment of RAD6/RAD18 and REV1. Monoubiquitination of PCNA facilitates recruitment of polη, poli, or polκ to synthesize 1-2 nt directly past the lesion. Extension by polζ leaves a suitable primer template for continued replication by polδ or polε.

Each of the Y-family enzymes is characterized by an open active site that is capable of accommodating damaged DNA, but the costs of this unique capacity are fidelity and processivity[59]. REV1 is required for TLS in eukaryotes[30;40], but appears to primarily play a structural role in recruiting other Y-family polymerases to actively bypass the damage[54;82]. Only 1-2 nt are inserted by polη, ι, or κ before they dissociate from the DNA, allowing extension by polζ[37;55], another critical TLS polymerase[40;51]. This extended primer terminus is sufficient for continued replication by polδ or ε. Although *in vitro* studies using purified enzymes have addressed the kinetics of bypassing specific lesions by individual polymerases, we designed this project to fill critical gaps in our knowledge of the cellular roles of Y-family polymerases.

The most striking role for TLS in humans is in xeroderma pigmentosum variant (XPV) syndrome. XP patients are extremely prone to UV-induced skin cancer because cells from most patients are deficient in proteins of the nucleotide excision repair (NER) pathway, which is responsible for recognizing and removing structurally diverse adducts that distort the DNA helix. However, cells from a subset of XP patients were found to be NER-proficient, despite the fact that the individuals displayed the classical XP phenotype[49;73]. These patients were found to be deficient in DNA polymerase η[35;44] and theirs are the most hypermutable known human cells in response to UV, despite the fact that NER actively repairs UV damage comparably to wild-type cells[77]. *In vitro* studies revealed that POLη replicates TT cyclobutane pyrimidine dimers, the most frequent lesions induced by UV, as faithfully as an undamaged TT template[36;43;44]. Hence, lack of POLη results in TLS past UV-induced lesions by more error-prone polymerases. Recently developed knockout mouse models have been used to examine the *in vivo* role of polη and poli in response to chronic UV exposure. *polη*$^{-/-}$ *poli*$^{+/+}$ mice were extremely susceptible to UV-induced skin cancer and their cells displayed a similar hypermutable phenotype to their human counterparts, validating the mouse model of

XPV[27;58]. Our group showed that fibroblasts from *polη*$^{-/-}$ *poli*$^{-/-}$ mice showed decreased UV-induced mutation frequency; however, these mice developed tumors about twice as fast as *polη*$^{-/-}$ *poli*$^{+/+}$ mice[27]. Deficiency in *poli* was also required for metastases and was associated with increased tumor burden[58]. These data suggest a role for polι as a tumor suppressor that is separate from its mechanism as a mutagenic polymerase, since deficiency in the enzyme decreased mutation frequency and yet increased tumor development and tumor burden. We will investigate the tumor suppressor role of polι in this project.

Recruitment of Y-Family Polymerases

Previous reports showed that while *POLη* deficiency in human cells drastically increased the UV-induced mutation frequency, no effect was seen on mutagenesis induced by benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE)[78]. In contrast, recent studies by our group have shown that BPDE-induced mutation frequency is *decreased* in *POLη*$^{-/-}$ human and murine fibroblasts (see References). These results are consistent with reports of error-prone bypass of BPDE damage using purified polη[87;88;92]. Along with the novel finding that polη acts in error-free bypass of UV damage and error-prone bypass of bulky BPDE lesions in live mammalian cells, our results suggest that the recruitment of Y-family polymerases to stalled replication fork is *not* dependent upon the lesion. Rather, we hypothesize that polη is the primary TLS polymerase and is recruited to stalled replication forks first, and in its absence polι or polκ would be allowed greater access to the lesion. We will examine this hypothesis in Chapter 2 using knockout models of polη and polι in response to treatment with chemically distinct genotoxicants.

DNA Damage and Cell Cycle Checkpoints

Environmental carcinogens such as benzo[a]pyrene, aminofluorene, and nitropyrene are metabolically activated and bind to DNA forming bulky, helix-distorting lesions[6;7;22;63;72]. These adducts are recognized and removed by nucleotide excision

repair[17;18;47;48]. However, if these adducts remain in the DNA when the cell enters S-phase they pose a significant threat to genomic integrity: the DNA replication complex cannot replicate past such adducts in the template. Although the proximal signals are poorly understood at the present time, stalled replication forks activate cell cycle checkpoints, presumably to allow removal of the adducts. Checkpoints are complex signal transduction pathways that block cell cycle progression in response to DNA damage[93]. These pathways exist for G_1, S, G_2 and M phases to manage potential DNA damage throughout the cell cycle; the intra-S checkpoint is most relevant to this thesis. The molecular mechanisms underlying the activation of this checkpoint are not well understood. Available data indicate that stalled replication forks initiate the S-phase checkpoint by recruitment of the protein kinase ATR, and to a lesser extent the related protein ATM. ATR recruitment is dependent on a structural protein (ATR interacting protein) and may be initiated by extensive regions of single stranded DNA coated by RPA[95;96]. The downstream targets of phosphorylation by ATR/ATM are myriad, and in this context are thought to promote degradation of cofactors that are required for progression through S-phase.

It is of interest that bulky adducts induced by BPDE elicit an S-phase checkpoint in human lung carcinoma cells[31;79] that is dependent upon the Y-family polymerase κ. A deficiency in this polymerase results in failure to recover from BPDE-induced S-phase arrest and causes double strand breaks, likely due to collapse of stalled replication forks[10]. This is the first direct evidence of cellular requirement for a translesion synthesis polymerase for cell cycle regulation, and supports the hypothesis that such polymerases are implicated in the activation of the intra-S-phase checkpoint. This hypothesis is strengthened by a recent report that human cells with significantly reduced expression of *REV7* showed a decreased rate of S-phase progression after UV irradiation[50]. REV7 is the non-catalytic partner of REV3 in the B-family polymerase

POLζ, the first discovered TLS polymerase[56]. In Chapter 3, we propose to examine the hypothesis that the tumor suppressor function of polι is due to a similar function of this polymerase in activating cell cycle checkpoints.

miRNA and Cancer

miRNA are 20-22 base RNAs which negatively regulate gene expression by destabilizing target mRNAs. Initial transcription of miRNA genes yields larger primary miRNAs (pri-miRNAs) which are cropped by nuclear enzymes into ~65nt hairpin-shaped precursors (pre-miRNAs). Pre-miRNAs are exported into the cytoplasm, where further processing yields a 22 nt duplex which complexes with the miRNA-induced silencing complex (miRISC). This complex can target other transcripts for degradation through perfect sequence complementarity in the 3' UTR of the target or, likely more common in animals, repress translation of targeted transcripts through indirect complementarity[3;14]. miRNA gene expression patterns are greatly altered in many cancers, and several miRNAs are known to target oncogenes or tumor promoters (reviewed in [86]), making them strong candidates for cancer studies. It has been shown by our collaborators that the ubiquitous carcinogen BPDE alters miRNA gene expression profiles in small airway epithelial cells (unpublished observations). We hypothesize that *poli* status will alter miRNA gene expression changes induced by other carcinogens, including UV, and that these changes will provide insight as to the pathways effected by *poli* proficiency.

Skin Cancer as a Tumorigenesis Model

More than one million new cases of nonmelanoma skin cancer (NMSC) will be diagnosed in the U.S. in 2007, making it the most commonly diagnosed cancer in the country (American Cancer Society 2007 Facts and Figures). Ultraviolet radiation is the most ubiquitous carcinogen in the environment and is the primary etiological agent causing NMSC. Carcinogenesis from environmental UV exposure can be attributed to radiation ranging from 280 – 320 nm in wavelength (UVB), which generates

photoproducts in DNA capable of causing mutations. Short-wave UV (UVC) ranges from 100 – 280 nm and is also highly carcinogenic, but is filtered out completely by atmospheric ozone and is thus not environmentally relevant. UVA is characterized by wavelengths between 320 – 400 nm and is only carcinogenic at much higher doses in mice compared to UVB due to absorption by cellular chromophores other than DNA and principle production of reactive oxygen species[9;52]. Ultraviolet radiation produces measurable effects in human and mouse skin. *Trp53* is frequently mutated in UV-induced mouse and human skin cancers[13;53], and this mutated tumor suppressor causes formation of precancerous clones in irradiated skin[8;38]. UV also induces a well characterized apoptotic sunburn response which appears to require wild type *trp53*. Sunburn cells are characterized by pycnotic nuclei and intensely eosinophilic cytoplasm, as well as DNA strand breaks[94]. We hypothesize that polι promotes p53 accumulation, cell cycle arrest, and apoptosis in its role as a tumor suppressor.

Xeroderma pigmentosum (XP) has been described as a syndrome characterized by extreme predisposition to UV-induced skin cancer. The majority of patients have dysfunctional NER, resulting in faulty recognition and repair of helix distorting lesions including 6-4 photoproducts and cyclobutane pyrimidine dimers (CPD) induced by UV. Patients from XP complementation group A display the most severe phenotype, including extreme photosensitivity and neurological defects, and typically die of metastatic skin cancer early in life. Hairless mice that are homozygous $xpa^{-/-}$, developed by Harry van Steeg, display a similar phenotype and are extremely susceptible to UV carcinogenesis[19] with a tumor latency of ~10 weeks as compared to ~1 year with Balb/c mice ([27] and unpublished observations). The van Steeg mice represent an excellent and well studied skin cancer model[19;21;26;66;74;75] and will be used in this thesis.

Benzo[a]pyrene (B[a]P) is also a well studied skin carcinogen. It is clear that benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), a metabolic derivative of B[a]P, is the

ultimate carcinogen[11;71]. BPDE formation proceeds through the action of CYP450, microsomal epoxide hydratase, and repeated CYP450 oxidation (reviewed in[62]). Carcinogenic activity of B[a]P is primarily due to *in vivo* binding of the ultimate carcinogen to DNA to form bulky adducts[71]. As analyzed by ^{32}P-postlabeling, stable BPDE-N^2-dG adducts constitute over 98% of adducts formed in mouse skin treated with anti-BPDE[16]. These adducts are normally recognized and repaired by enzymes in the NER pathway, and the NER-deficient mice we propose to use have been shown to accumulate more BPDE:DNA adducts than wild-type mice after B[a]P treatment[20]. These mice are also more sensitive to B[a]P-induced lung carcinogenesis[33], and represent an excellent model for chemical carcinogenesis.

Acetylaminofluorene (AAF) is a well characterized model mutagen and liver, intestine, and urinary bladder carcinogen. AAF is subject to numerous metabolic activities *in vivo* that can result in formation of electrophilic intermediates capable of binding to DNA. N- and O-acetylation, hydroxylation, and sulfation all may lead to activation of the compound through many possible pathways[5]. Three major adducts are formed by AAF *in vitro* and *in vivo* and serve as NER substrates. AAF-C8-dG and AF-C8-dG form at differing ratios depending on cell type and activity of microsomal deacetylase[63]. AF-N2-dG is formed as a minor but persistent adduct *in vivo*[80] and is significantly less mutagenic than C8-dG adducts[84]. In mouse epidermal cells treated with the active metabolite N-acetoxy-2-acetylaminofluorene (AAAF), AF-C8-dG adducts make up over 90% of DNA adducts formed[63]

Significance

Our studies support a role for mouse polι in mutagenic translesion synthesis and in tumor suppression after UV exposure. In addition, limited available evidence indicates there may be differences in Y-family polymerase activities in response to UV and chemical damage. These areas represent critical gaps in our knowledge of mammalian

mutagenesis. We hypothesize that DNA polymerase ι acts as a mutagenic polymerase in translesion synthesis and as a tumor suppressor through a separate mechanism. We propose to investigate the cellular roles of Y-family polymerases in order to further understanding of the mechanism of carcinogenesis by environmental agents with the ultimate aim of preventing cancer caused by these agents.

CHAPTER 2

EVALUATE THE MUTAGENIC PROPERTIES OF CHEMICAL CARCINOGENS IN MAMMALIAN FIBROBLASTS DEFICIENT FOR Y-FAMILY POLYMERASES

INTRODUCTION

In this Chapter we will compare the cytotoxic and mutagenic properties of BPDE and AAF. BPDE is the ultimate genotoxicant metabolized from the ubiquitous carcinogen benzo[a]pyrene and primarily forms N^2-dG adducts[15;69]. This carcinogen will be used in Chapter 4 for *in vivo* tumorigenesis studies. AAAF is the active metabolite of AAF and primarily adducts C8 of deoxyguanosine[57]. This Chapter will allow us to examine the hypothesis that structurally different chemical adducts have varying mutagenic activity when bypassed by Y-family polymerases. We will also directly test the somatic mutation hypothesis of cancer by correlating *in vitro* mutagenesis data with *in vivo* tumorigenesis data collected in Chapter 4.

MATERIALS AND METHODS

Cell lines to be used

We used established NF1604, XP4BE, and XP115LO human cell lines as well as primary fibroblasts from knockout mice. NF1604 cells were isolated from normal fetal lung tissue, and XP4BE and XP115LO cells are from XP variant patients. All lines are stably transfected with a plasmid expressing human telomerase reverse transcriptase. This gives them an infinite lifespan, but they are otherwise normal[60]. *polη* knockout mice were recently generated by our collaborators in a C57Bl/6 hybrid background[42]. To generate *polη*$^{-/-}$*poli*$^{-/-}$ double knockouts, these animals were bred with 129/Ola mice, which have been shown to carry a nonsense mutation in *poli* resulting in a protein truncated at Ser-27[45]. Primary fibroblasts have been isolated from these mice and used for mutagenesis studies in our laboratory[27], and these cells were used in this Chapter. Mouse fibroblasts were grown in monolayer cultures using α-MEM supplemented with non-essential amino acids, penicillin-streptomycin and 10% fetal bovine serum. They were maintained in exponential growth. We routinely grow primary murine cells in a 3% O_2/5% CO_2/92% N_2 atmosphere[61].

hprt mutagenesis

For genotoxicant treatment, cells were plated at 1 x 10^4 cm^{-2}. The culture media was removed and cells were washed twice with sterile phosphate-buffered saline (PBS, pH 7.4). Serum-free media was added to the cells along with each chemical carcinogen for a period of one hour. Doses of 0 – 250 nM BPDE and 0 – 2.0 μM AAF were used for cytotoxicity studies. We used the dose giving ~37% survival for mutagenesis studies. After one hour of treatment, the culture media was aspirated followed by two washes in PBS. For cytotoxicity, cells were trypsinized and counted, then replated at cloning density (the exact number depends on the expected survival). After two weeks, plates

were stained with 1% crystal violet and counted. For mutagenesis, cells were maintained in exponential growth for eight days after treatment, after which we trypsinized, counted, and selected 1 X 10^6 cells with 40 μM 6-thioguanine at a density of 3000 cm^{-2}. After 2 weeks of selection, we isolated individual TG^r clones, trypsinized, and resuspended them in RNase-free PBS. The technique for amplifying the *hprt* gene was performed as described[83] with modifications routine in our laboratory[23]. Briefly, TG^r colonies were pelleted at 4℃ and the PBS removed. The cells were directly lysed and cDNA generated using a cDNA cocktail. After incubation at 37℃ for 1 h amplification was performed using a Taq polymerase cocktail and two outer primers. The 5' primer is from position -47 to -28 relative to the start codon (GGC TTC CTC CTC AGA CCG CT). The 3' primer is from position 790 to 771 (ACA TCA ACA GGA CTC CTC GT). Reamplification using an inner primer set (5', from -27 to -8, TTT TGC CGC GAG CCG ACC GG and 3', from 770 to 751, ATT TGC AGA TTC AAC TTG CG) will follow. The PCR products were sequenced with each of the inner primers using an ABI 370 automated DNA sequencer and dRhodamine dye terminator chemistry.

RESULTS

BPDE in Human Fibroblasts

We treated *POLη*$^{-/-}$ XP115LO and wild type NF1604 human fibroblasts with 150 or 200 nM BPDE and measured clonogenic survival and mutagenesis. These cells are isolated from an XP variant patient (XP115Lo) and normal fetal lung (NF1604) and are stably transfected with a plasmid expressing human telomerase reverse transcriptase. This gives them infinite lifespan, but they are otherwise normal[46;76]. After treatment and eight days of expression, cells were selected with 6-thioguanine (TG). Table 1 shows that XP variant fibroblasts are moderately sensitive to killing by BPDE, which is similar to the phenotype displayed by XP variant cells in response to UV[49]. However, unlike XPV cells after UV irradiation, XP115LO fibroblasts showed *decreased* mutation frequencies when compared to wild type NF1604 fibroblasts (Table 1). This novel result has been reproduced over three experiments using these cells and is consistent with published reports of *in vitro* error-prone TLS by purified human POLη past BPDE-N^2-dG DNA adducts[87;88;92]. Our results indicating POLη in error-prone TLS of BPDE lesions contrasts with the known role of POLη in error-free TLS past UV-induced lesions. Our conclusion that POLη acts in error-prone bypass of BPDE lesions is also in sharp contrast with a report that POLη is unresponsive to BPDE lesions in human *POLη*$^{-/-}$ XP4BE cells[77]. We amplified and sequenced the *HPRT* gene from mutant colonies and found very little difference in the mutation spectrum, e.g. the types of mutations induced, of wild type NF1604 and *POLη*$^{-/-}$ XP115LO fibroblasts (Table 2). The similarity in BPDE-induced base substitution indicates that the enzyme acting to bypass the BPDE lesion in the absence of POLη has a similar mutation spectrum for this adduct.

BPDE in Murine Fibroblasts

To extend the results we obtained from studies of human cells, we performed similar experiments with primary murine fibroblasts deficient for polη, poli, or both. We first compared the sensitivity to BPDE of the cells using *in situ* cytotoxicity tests. For this method, cells were plated at cloning density and treated with varying doses of BPDE. Figure 2.1 displays the results of one experiment which show no apparent differences in the cytotoxicity of BPDE among the four cell lines, although significant sensitivity of *polη*$^{-/-}$ *poli*$^{-/-}$ double knockout cells may be shown after experimental replication. The dose resulting in 37% survival (D_{37}) for each cell line was ~100 nM. It is known from unpublished observations that mouse primary fibroblasts are more sensitive to genotoxicant treatments at low densities. It is for this reason that further cytotoxicity studies are performed by treating cells at 1 x 10^4 cells/cm^2 and immediately replating at cloning density. This method of replating cytotoxicity accurately reflects the survival of cells treated at equal density for mutagenesis. Figure 2.2 shows the results of one or two replating cytotoxicity studies using 150 nM BPDE, the known D_{37} of wild-type primary murine fibroblasts at replating density. As in Figure 2.1, there are no obvious differences in sensitivity to BPDE treatment with the possible exception of the double knockout cells. Significantly, we used heterozygous *polη*$^{+/-}$ *poli*$^{+/+}$ cells for experiments shown in Figures 2.2 and 2.3. These cells were provided by a collaborator as homozygous *polη* knockouts and were genotyped for confirmation of that only after these studies were completed. We have since verified by PCR all genotypes indicated in this thesis. Strikingly, haploinsufficiency for *polη* in these cells was sufficient to markedly reduce the mutation frequency induced by 150 nM BPDE as shown in Figure 2.3. These results support our studies of human cells indicating POLη in error-prone bypass of BPDE DNA adducts in contrast to its role in error-free bypass of UV damage. Similarly, *polη*$^{+/+}$ *poli*$^{-/-}$ fibroblasts showed drastically reduced mutation frequencies after

BPDE treatment, and *polη*$^{-/-}$ *poli*$^{-/-}$ cells exhibited a 98% reduction in *hprt* mutations induced by 150 nM BPDE. These results reflect the findings of *in vitro* studies which have characterized the error-prone bypass of BPDE-N^2-dG DNA adducts, the major adduct induced by this carcinogen, by purified POLη and POLι[67]. We hypothesize that the low mutation frequency of *polη*$^{-/-}$ *poli*$^{-/-}$ double knockout murine cells is due to error-free lesion bypass by polκ, which has also been characterized *in vitro*[90;91].

AAAF in Murine Fibroblasts

We examined the cytotoxic effects of N-acetoxy-2-acetylaminofluorene (AAAF) using replating cytotoxicity assays. This well characterized mutagen is a major metabolite of acetylaminofluorene, a carcinogen present in diesel exhaust, and predominantly forms AF-C8-dG adducts[63;64]. This lesion is structurally distinct from the N2-dG adduct which predominates after BPDE treatment, and the differences in cytotoxicity of the two chemicals are readily apparent in Figure 2.4. As compared to the very similar sensitivities of these cells to treatment with BPDE, *polη* and/or *poli* deficient murine fibroblasts appear to be more sensitive to killing by AAAF. The D_{37} calculated from these studies varies from 0.83 μM for wild type cells to 0.16 μM AAAF for *polη*$^{-/-}$ *poli*$^{-/-}$ double knockout cells. This pattern of increased sensitivity is very similar to that characterized by our group after UV treatment of these cells[27], and supports a role for these enzymes in prevention of replication fork collapse and double strand break cytotoxicity. We hypothesize based on *in vitro* studies of purified yeast polη[85] and human POLι[89] that polη acts in error-free bypass of AAAF-induced DNA damage, while poli participates in error-prone TLS of the lesion. We will investigate this lesion-specific fidelity of cellular polη and poli further in Chapter 4.

Experiment		1	2	3
BPDE Dose (nM)		150	150	200
Percent Survival	1604	73	62	33
	XP115Lo	53	55	21
Induced Mutants / 10^6 Clonable Cells	1604	263	208	483
	XP115Lo	53	85	104

Table 2.1. Cytotoxicity and mutagenesis of BPDE in human cells. Transformed human fibroblasts from normal (1604) or XP variant (XP115Lo) patients were treated with 150 or 200 nM BPDE. XP115Lo cells appear to be slightly more sensitive to the treatment and are hypomutable.

	1604	XPV
Transitions	**8.3**	**8.7**
G>A	8.3	8.7
Transversions	**91.7**	**91.3**
G>T	58.3	69.6
G>C	8.3	17.4
A>T	25.0	4.3

Table 2.2. BPDE-induced *HPRT* point mutations in human fibroblasts. Thioguanine-resistant clones were isolated after BPDE treatment. *HPRT* mRNA was reverse transcribed and amplified by PCR for sequencing. Numbers represent percentage of total point mutations.

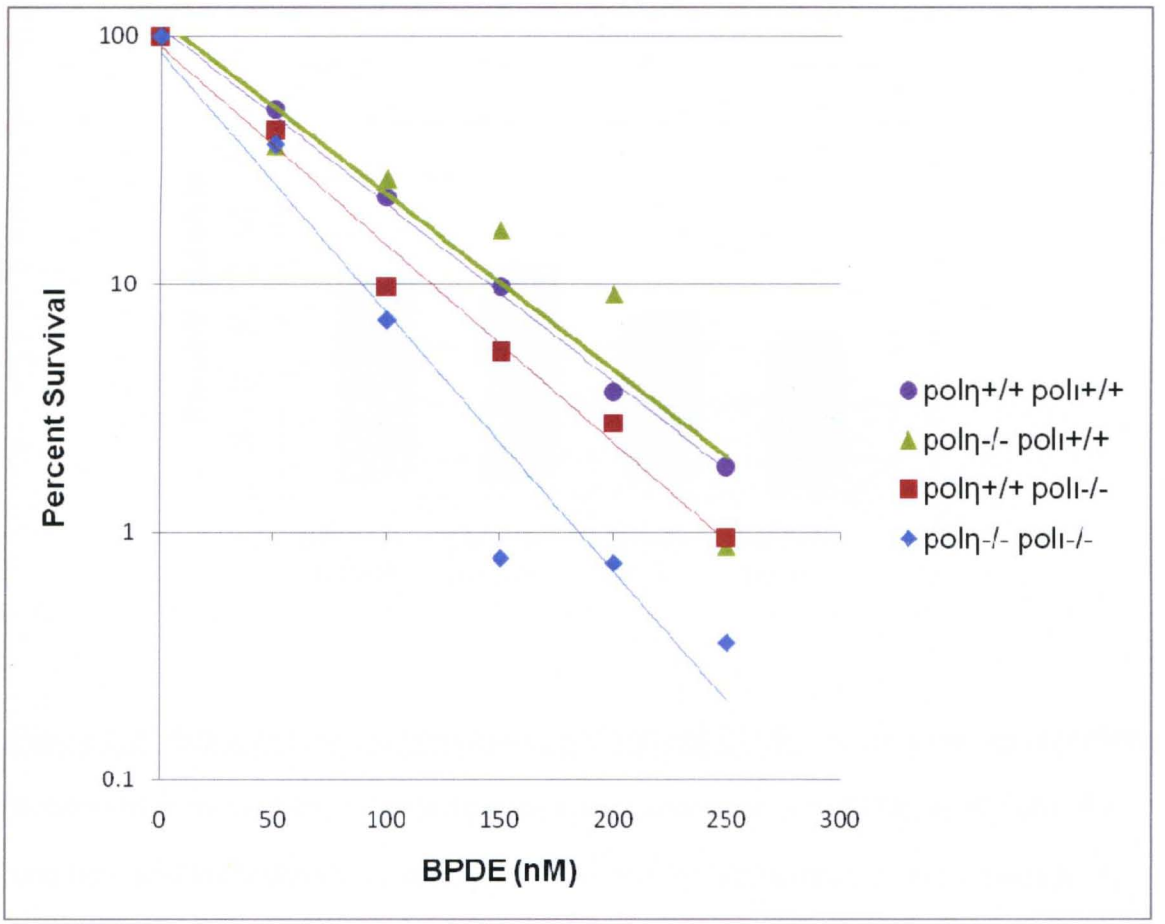

Figure 2.1. Survival of murine fibroblasts after BPDE. Primary dermal fibroblasts isolated from mice of the indicated genotypes were treated with BPDE at cloning density and refed after one week. After two weeks, colonies were stained with crystal violet and counted to assay survival. It should be noted that these cells are more sensitive to chemical genotoxicants when treated at low density.

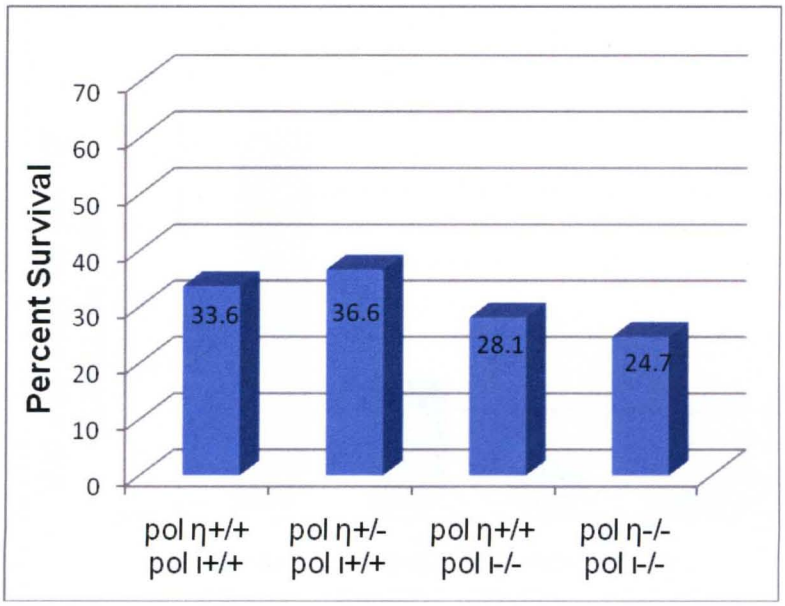

Figure 2.2. Suvival of murine fibroblasts after 150 nM BPDE. Primary dermal fibroblasts isolated from mice of the indicated genotypes were treated with BPDE at 10^{-4} cm^{-2} for one hour and immediately replated at cloning density. Note that a *polη* heterozygote was used.

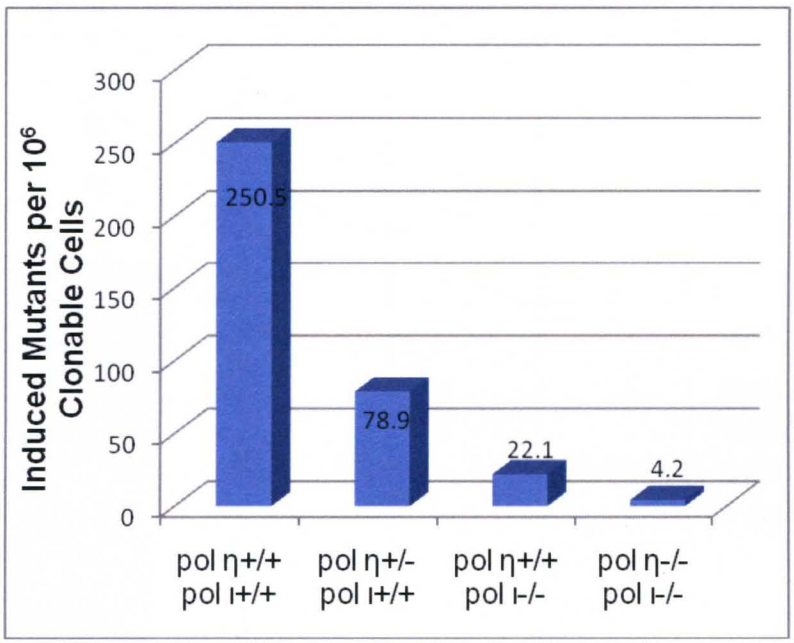

Figure 2.3. Mutagenesis after 150 nM BPDE. Primary dermal fibroblasts isolated from mice of the indicated genotypes were treated with 150 nM BPDE at 10^4 cm^{-2}. After eight days of exponential growth, cells were replated in 40 μM 6-thioguanine and grown for two weeks. Thioguanine-resistant clones were stained with crystal violet. Note that a *polη* heterozygote was used.

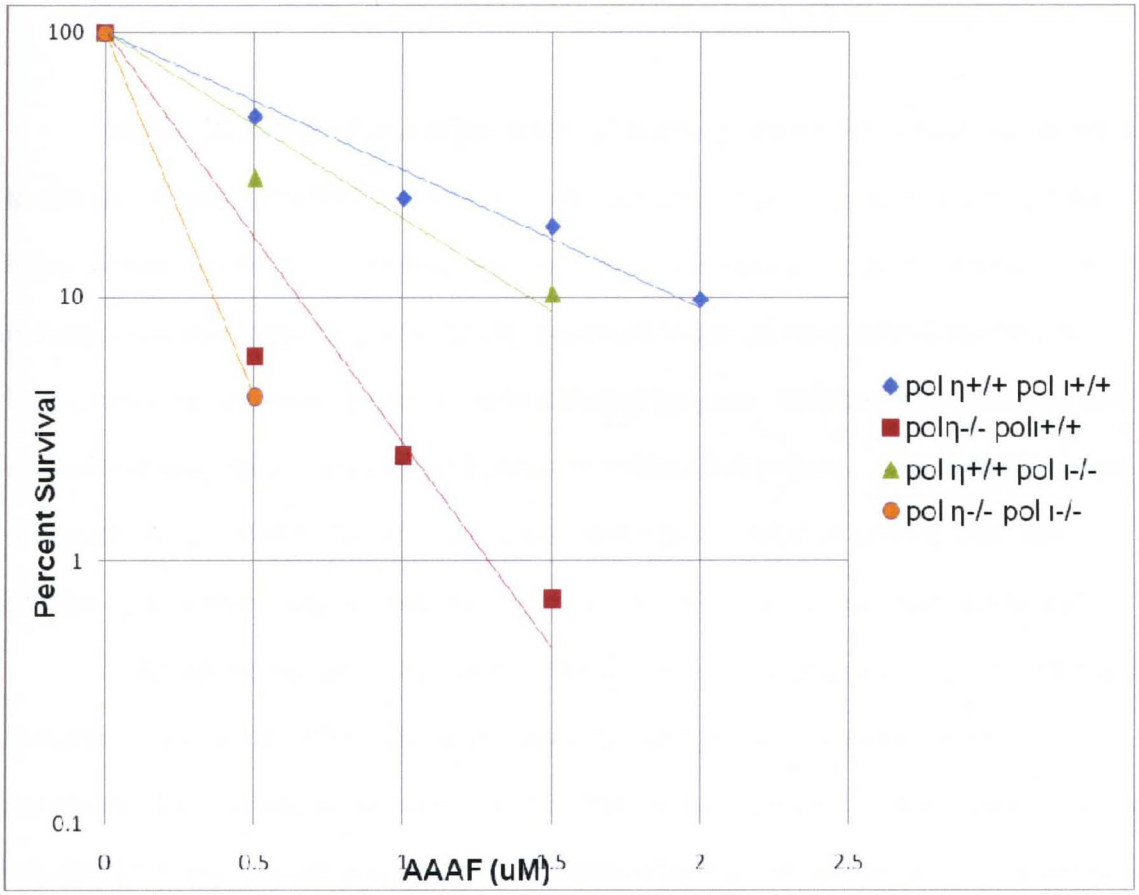

Figure 2.4. Cytotoxicity of AAAF in murine fibroblasts. Primary dermal fibroblasts isolated from mice of the indicated genotypes were treated with AAAF at 10^4 cm^{-2} for one hour and immediately replated at cloning density. Cells were refed after one week and stained with crystal violet after two weeks.

CONCLUSIONS AND FUTURE DIRECTIONS

We conclude from these initial data that neither polη nor polι is likely to contribute to cellular survival after BPDE treatment, although loss of polη appears to make cells more sensitive to AAAF. Strikingly, we found that both *polη* and *polι* deficiency caused reduced mutation frequency after BPDE treatment in our primary dermal fibroblasts. This effect was also seen in human cells deficient for *polη*. This supports a role for both enzymes in error-prone, mutagenic bypass of BPDE:DNA lesions. This is confirmation of results using purified polη and polι, but in contrast to published reports that *polη* deficiency in human cells causes no change on BPDE-induced mutation frequency[77].

AAAF causes structurally distinct DNA lesions and appears to be more cytotoxic to murine cells deficient for *polη*. *polι* status did not appear to modulate AAAF sensitivity. These results are similar to the effect of deficiency for these enzymes on UV sensitivity[27], and we will investigate mutagenesis by this carcinogen at the *hprt* locus as we have for BPDE. We hypothesize based on in vitro experiments[85;89] that polη participates in error-free bypass of AAAF damage and that polι is active in error-prone bypass of these lesions, again similar to the known roles of these enzymes in UV damage bypass[27].

CHAPTER 3

INVESTIGATE THE TUMOR SUPPRESSOR ROLE OF MURINE POL IOTA

INTRODUCTION

We have shown that *poli* deficiency in a *polη*$^{-/-}$ background causes decreased UV-induced mutation frequency and decreased UV-induced tumor latency[27], indicating a possible tumor suppressor role for the enzyme. It has been shown that *polκ*$^{-/-}$ mouse fibroblasts arrest in S-phase and display increased checkpoint signaling after treatment with BPDE and that recovery from this checkpoint is dependent upon polκ. We propose to investigate the tumor suppressor role of polι in this Chapter, specifically focusing on cell cycle checkpoint signaling. We will also investigate differential miRNA and global gene expression responses to UV damage to determine the regulation of other genes and pathways by polι.

MATERIALS AND METHODS

Irradiation protocol

Cells were plated at cloning density (the exact number depends upon the expected survival) or 1 x 10^4 cm^{-2} and allowed to attach overnight. The culture medium was then be aspirated and cells will be exposed to 1 – 20 J/m^{-2} UVC. Culture medium was immediately replaced and cells were refed at one week and stained for counting and cytotoxicity determination at two weeks or harvested at various time points for analyses detailed below.

Global gene expression

In order to identify potential effects of polι expression on a larger scale, we examined the effects on global gene expression due to loss of *polη*, *polι*, or both in primary murine fibroblasts. This experiment was designed to discover the effect of polι deficiency on the expression of genes in known tumor suppressor or promoter pathways. Cells were irradiated with 20 Jcm^{-2} at 1 x 10^4 cm^{-2} and total RNA was extracted using the Qiagen miRNeasy kit. After amplification, resultant cDNA was transcribed using the GeneChip® IVT Labeling Kit for hybridization on Affymetrix MOE 430_2.0 arrays using a GeneChip® Scanner 3000 7G. Statistical analysis (ANOVA) of the microarray data was performed to evaluate significant changes between the groups with a q-Value <0.05 using FDR (False Discovery Rate) multiple testing correction and Partek 6.1 software. We examined expression patterns associated with UV treatment, as well as differences in UV response between all cell lines. We randomly verified results using quantitative real-time PCR and Western Blots for affected proteins.

miRNA microarrays

To investigate the effect of *polι* deficiency on cancer-regulating miRNA expression, we analyzed global miRNA gene expression using a microarray-based

assay (LC Sciences). Total RNA samples isolated before and after UV treatment were enriched for miRNA, labeled with Cy3 or Cy5, and hybridized to a dual-color microarray chip. For this technique, the relative abundance of each miRNA in each sample was analyzed by the proportion of Cy3/Cy5 fluorescence at each probe site on the chip. Each chip contains six redundant probes for each of the known 568 mature mouse miRNAs. After background subtraction and data normalization, statistical correlation between the three chips representing independent biological replicates identified any miRNA genes altered between groups. As above, we analyzed data for UV-responsive miRNA and differences in UV response between cell lines. Results were randomly validated using Northern Blots and RT-PCR.

RESULTS

To determine the potential mechanism by which polι acts as a tumor suppressor in mammalian cells, we examined UV-induced changes in global gene expression in *polη*$^{-/-}$ *polι*$^{+/+}$ and *polη*$^{-/-}$ *polι*$^{-/-}$ primary murine fibroblasts. Our group previously showed that *polη*$^{-/-}$ *polι*$^{-/-}$ fibroblasts get fewer mutations after UV exposure, but mice with this genotype have a decreased UV-induced tumor latency compared to the XPV model *polη*$^{-/-}$ *polι*$^{+/+}$ mice[27]. To examine the effect of *polι* deficiency on global gene expression, we plated cells at 1 x 10^4 cm^{-2} 24 hr before treatment with 4 Jm^{-2} UVC. mRNA was isolated 24 hr after treatment, reverse transcribed into cDNA and amplified for global gene expression using Affymetrix Mouse Genome 430 2.0 GeneChip® arrays. For data analysis, we used a 2-way ANOVA incorporating cell line and treatment as well as their interaction as factors. The resulting p-Values were false discovery rate (FDR) corrected and cut off at a corresponding q-value of 0.05. Table 3 shows the 19 genes whose expression was modulated in different ways by treatment with UV between *polη*$^{-/-}$ *polι*$^{+/+}$ and *polη*$^{-/-}$ *polι*$^{-/-}$ cells. For example, protein tyrosine phosphatase receptor type V (*ptprv*) was the gene that showed the most statistically significant differential expression in the cell lines after UV exposure. In *polη*$^{-/-}$ *polι*$^{+/+}$ cells, UV induced a 1.56-fold upregulation of ptprv, while a 5.67-fold UV-induced upregulation was observed in *polη*$^{-/-}$ *polι*$^{-/-}$ cells. This gene has only recently been characterized[41], but has already been linked to p53 signaling, cell cycle checkpoints, and tumor suppression[24;25]. *ADAM8* (A Disintegrin And Metalloprotease 8) has been shown to be a possible diagnostic marker for lung cancer and renal cell carcinoma in humans[34;68] and is negatively correlated with prognosis in prostate, renal cell, and brain cancers[29;81]. Also relevant to this project is *cdc20*, which promotes progression through and exit from M phase and is often

overexpressed in malignancies[2;39]. These genes will provide an important basis for the investigation of the tumor suppressor role of polι.

We also examined the effects of UV treatment on miRNA expression in these cells. Total RNA isolated after the treatment described above was enriched for miRNA and labeled with Cy3 or Cy5. Samples were hybridized in oppositely labeled pairs to two identical dual-color microarray chips containing probes for the 568 known mature miRNAs from the Sanger Database v10.0. Figure 3.1 shows results after background subtraction and data normalization. Expression of the miRNA listed was modulated after UV treatment. To our knowledge, this is the first evidence of altered miRNA expression after UV radiation in mammals. Using bioinformatics tools including the Sanger miRNA database for target mRNA prediction, we will be able to correlate miRNA expression data from all four cell lines to discover commonly affected tumor suppressor and promoter pathways altered by *polι* deficiency.

Name	Description	p-value	Fold Change ($pol\eta^{-/-}$ $poli^{+/+}$)	Fold Change ($pol\eta^{-/-}$ $poli^{-/-}$)
PTPRV	protein tyrosine phosphatase, receptor type, V (pseudogene)	0.010	1.56	5.674
ADAM8	ADAM metallopeptidase domain 8	0.016	-1.01	1.557
PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1	0.018	2.21	24.246
KIF23	kinesin family member 23	0.019	1.98	1.349
SLC9A3R1	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	0.022	-1.18	1.229
ODZ3	odz, odd Oz/ten-m homolog 3 (Drosophila)	0.023	-1.11	-3.631
PRKG1	protein kinase, cGMP-dependent, type I	0.026	-2.34	-4.339
C8ORF4	chromosome 8 open reading frame	0.037	2.69	1.362
GSTO1	glutathione S-transferase omega 1	0.041	1.33	1.091
CDC20	cell division cycle 20 homolog (S. cerevisiae)	0.041	1.78	1.422
PC	pyruvate carboxylase	0.042	1.28	1.697
DGKA	diacylglycerol kinase, alpha 80kDa	0.042	1.52	2.467
RAD54L	RAD54-like (S. cerevisiae)	0.042	1.63	1.308
SLC9A3R2	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 2	0.044	-1.03	1.433
CRYAB	crystallin, alpha B	0.045	2.80	1.678
EFS	embryonal Fyn-associated	0.045	2.83	5.238
PAIP1	poly(A) binding protein interacting protein 1	0.048	1.30	-1.255
KCNIP1	Kv channel interacting protein 1	0.048	-1.30	-3.485
MAN1A1	mannosidase, alpha, class 1A, member 1	0.050	1.12	1.398

Table 3.1. Differentially regulated, UV-responsive transcripts in primary murine fibroblasts. Dermal fibroblasts were treated with 4 Jm^{-2} and total RNA was isolated after 24 hr. Affymetrix Mouse Genome 430 2.0 GeneChip arrays were used to quantify mRNA levels. UV-responsive mRNAs were identified using 2-way ANOVA incorporating cell genotype and treatment, as well as their interaction, as factors. The 19 genes shown here were differentially regulated by UV treatment between cells of the two genotypes.

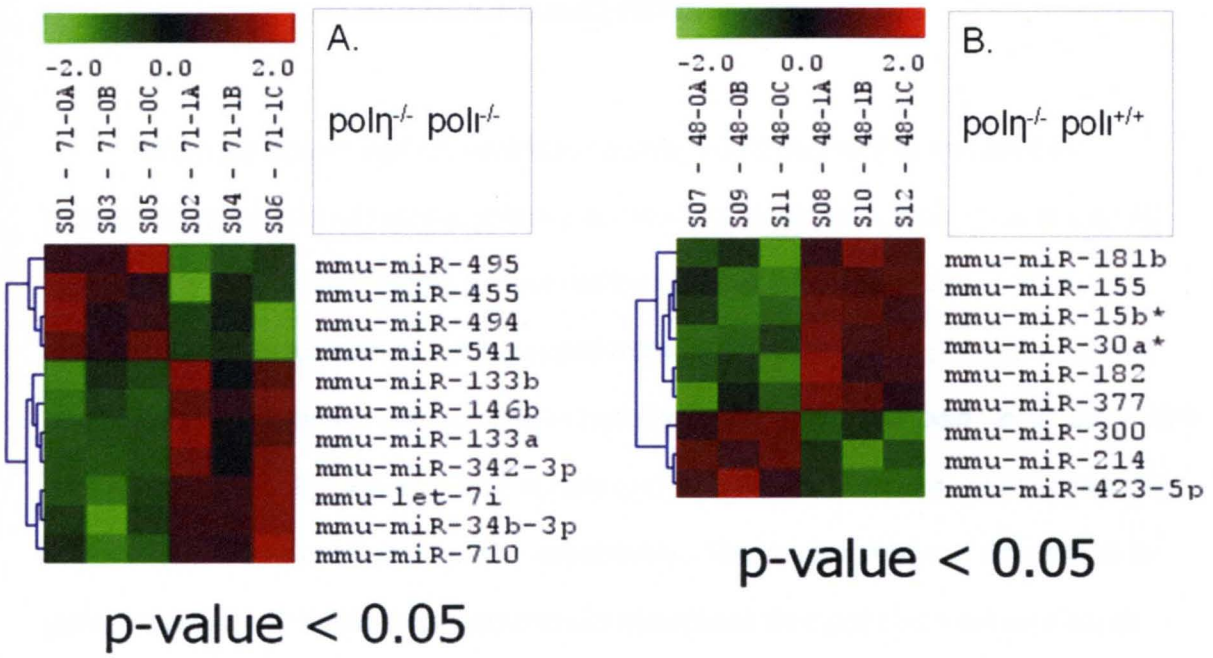

Figure 3.1. UV-responsive miRNA. Primary dermal fibroblasts were treated with 4 Jm^{-2} and total RNA was collected after 24 hr. After enrichment for small RNA, samples were labeled with either Cy3 or Cy5 and hybridized in oppositely labeled pairs to two identical dual-color microarray chips containing probes for mature murine miRNA from the Sanger Database v10.0. Two-way ANOVA was used to identify miRNAs with modulated expression after UV.

CONCLUSIONS AND FUTURE DIRECTIONS

We have shown that UV radiation causes modulated mRNA and miRNA expression in our model system, primary dermal fibroblasts from mice. This is the first report of UV-responsive miRNA in mammalian cells. We have also shown that *poli* deficiency modulates both global gene and miRNA expression. We defined 19 genes that displayed differential UV modulation between *polη*$^{-/-}$ *poli*$^{+/+}$ and *polη*$^{-/-}$ *poli*$^{-/-}$ cells. We also defined mutually exclusive lists of nine and 11 miRNAs with modulated expression in *polη*$^{-/-}$ *poli*$^{+/+}$ and *polη*$^{-/-}$ *poli*$^{-/-}$ cells, respectively. The readily apparent differences in gene expression in these cells supports our hypothesis that *poli* could act as a tumor suppressor by modulating gene expression. To further investigate this hypothesis we will validate our initial results using qRT-PCR and western blotting for target protein levels.

In order to investigate cell cycle checkpoint activation after UV and the effects of *poli* deficiency, we will treat cells derived from mice deficient for polη, poli, or both with UV and utilize FACS analysis of cell cycle progression. We will also examine levels of checkpoint proteins and their phosphorylated epitopes via westen blot to detect activation of G1/S and G2/M checkpoints after UV treatment. These will include chk1 and chk2 along with phosphorylated epitopes chk1-phopho-Ser345 and chk2-phospho-Thr68. We hypothesize that *poli* deficiency will promote cell cycle progression after UV damage, thereby promoting increased mutagenesis and/or chromosomal instability and promoting transformation of *poli*$^{-/-}$ cells.

CHAPTER 4

CHARACTERIZE THE EFFECT OF POL IOTA DEFICIENCY ON *IN VIVO* SKIN TUMORIGENESIS USING A NOVEL MOUSE MODEL

INTRODUCTION

In this Chapter we propose to generate a novel mouse model for TLS-mediated UV- and B[a]P-induced skin cancer. This model is ideally suited for the hypothesis that polι acts as a tumor suppressor *in vivo* as we know that 100% of control SKH1/hairless *xpa*$^{-/-}$ mice will develop tumors after UV and chemical treatment. We hypothesize that deficiency in *polι* will result in decreased tumor latency after UV irradiation and B[a]P carcinogenesis. Combined sunburn cell quantification and TUNEL assay will allow us to compare apoptosis in the epidermis of UV-irradiated mice. We hypothesize that *polι* deficiency will result in decreased levels of p53 and p21 activation and consequently decreased apoptosis, providing the basis for a tumor suppressor mechanism. We will also compare *in vitro* mutagenesis data using BPDE from Chapter 2 with data from the B[a]P tumorigenesis experiment outlined in this Chapter. This will allow us to directly test the somatic mutation hypothesis of cancer and investigate chemical-induced tumor suppression by *polι*.

MATERIALS AND METHODS

Mouse Model

Previous studies used a combinatorial *polη* and *poli* knockout mouse model in a mixed 129/Ola X C57Bl/6 background. This model is not ideal for UV carcinogenesis studies for two reasons. First, the mice must be shaved twice weekly for UV treatment. Second, neither wild type nor $pol\eta^{+/+}$ $poli^{-/-}$ mice develop tumors after 20 weeks of UV treatment. For this reason, we are backcrossing these mice into an SKH1/hairless $xpa^{-/-}$ background. These mice were developed by Harry van Steeg and develop tumors after as few as 6 weeks of UV with an average latency of as little as 10 weeks[19]. The UV-induced cancer-prone phenotype of these mice will be an ideal background for elucidating the difference in skin cancer susceptibility between wild type and $pol\eta^{+/+}$ $poli^{-/-}$ mice, which is critical for our hypothesis that poli acts as a tumor suppressor *in vivo*. To breed the mice, we back crossed C57Bl/6 $pol\eta^{-/-}$[42] and 129X1/SvJ (JAX), which are naturally $poli^{-/-}$[45], with SKH1/hairless $xpa^{-/-}$ mice. We monitored the hairless (*hr*) locus via screening for hairless pups at 4 weeks of age. We monitored *xpa*[21], *polη*[42], *and poli*[45] using published PCR-based screens and genomic DNA from tail snips. All mice from generation F2 will be screened for $hr^{-/-}$ $xpa^{-/-}$ status as well as heterozygosity for the polymerase gene (*polη* or *poli*) and backcrossed into the SKH1/$xpa^{-/-}$ background for at least six generations[70]. Double knockout mice will be bred by crossing homozygous F7 $pol\eta^{-/-}$ $poli^{+/+}$ and $pol\eta^{+/+}$ $poli^{-/-}$ mice to generate $hr^{-/-}$ $xpa^{-/-}$ $pol\eta^{+/-}$ $poli^{+/-}$ animals. Doubly heterozygous littermates will be mated to obtain homozygous knockouts at all four loci. We have F2 *polη* mice and F2 *poli* mice in hand and expect to complete back crosses by fall 2008.

UV Irradiation protocol

A control group of 40 SKH1/hairless xpa$^{-/-}$ mice were segregated according to gender to prevent breeding over the course of the experiment and were housed in groups of five. The UV-B irradiation source is a bank of seven fluorescent FS40 T12 UV-B sources (Westinghouse) that is placed ~40 cm directly above the cages. (Pictures of the UV bed are included in Section F, Vertebrate Animals.) These lamps have a spectrum of 250-400 nm, peaking at ~310 nm. The lamps were shielded with Kodacel, which removes any short wave UV below 280 nm. The fluence wass determined at 313 nm using an International Light Radiometer with UVB-1 filter. The dorsal dose to the mice was 200 J/m^2 per week, delivered in three exposures per week. This dose has been determined as the lowest exposure level causing mild erythema (the minimal erythema dose, MED) in our laboratory (unpublished observations). However, if the mice develop erythema, the UV fluence to all groups will be reduced. After 4, 6, 8, or 10 weeks of such exposure, the UV irradiation was discontinued. The mice were examined weekly for tumor development and tumors were confirmed by two observations in which the volume of the tumor had increased. The mice were sacrificed if a tumor reached 10% of the animal's body mass. All mice were sacrificed 20 weeks after UV treatment initiation.

RESULTS

To determine the total UV dose necessary to ensure 100% of treated mice get tumors, we divided 20 male and 20 female SKH1/hairless *xpa*$^{-/-}$ mice into four groups. Each group had five males and five females and was housed in groups of five segregated by sex. We irradiated the mice with a dorsal dose of 66 Jm^{-2} UVB three times weekly. Group one was irradiated for four weeks (total dose ~800 Jm^{-2}), group two for six weeks (total dose ~1,200 Jm^{-2}), group three for 8 weeks (total dose ~1,600 Jm^{-2}), and group four for 10 weeks (total dose ~2,000 Jm^{-2}). One hundred percent of group three mice developed tumors with an average latency of ~12 weeks (Figure 4.1). Accordingly, we decided to use the 1,600 Jm^{-2} cumulative dose over six weeks of UV treatment for further experiments.

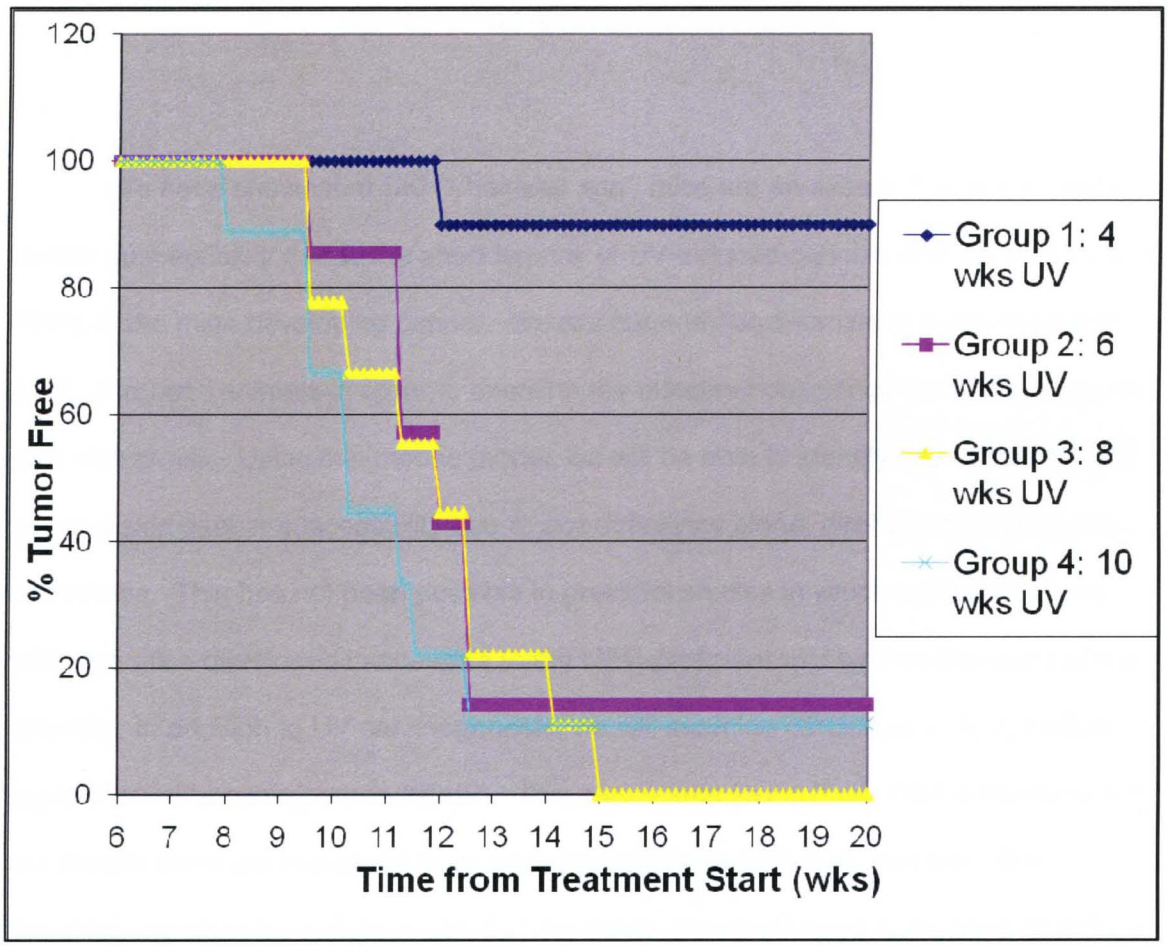

Figure 4.1. Tumor latency of SKH1/hairless *xpa*$^{-/-}$ mice. Four groups of ten mice each were treated with 200 Jm^{-2} for the time periods indicated. Tumor growth was monitored weekly and mice were killed 20 weeks after treatment initiation.

Conclusions and Future Directions

We have shown that SKH1/hairless *xpa*$^{-/-}$ mice are an excellent model for skin cancer susceptibility due to the short latency of UV-induced cancers and the likelihood of 100% of the mice developing cancer. We are currently back-crossing these mice with *polη*$^{-/-}$ and *poli*$^{-/-}$ animals in order to examine the effects of deficiency for these genes on skin cancer risk. Using this mouse model, we will be able to identify any effects on UV-induced skin cancer susceptibility due to *poli* deficiency alone, directly addressing our hypothesis. This has not been possible in previous studies in which neither *poli*$^{-/-}$ nor wild-type mice developed cancer due to the NER-proficient wild type background of the animals. In addition to UV carcinogenesis, we will examine carcinogenesis by topical application of benzo[a]pyrene (B[a]P). This carcinogen forms bulky DNA adducts which our results show are bypassed in an error-prone way by both polη and poli. We therefore hypothesize that mice deficient for these genes will have decreased incidence of B[a]P-induced skin cancer.

In addition, we will examine the affects of acute UV and B[a]P exposure on the skin of these mice through histological analysis and immunohistochemical staining. By analyzing appearance of apoptotic and p53-positive cells, in addition to using the TUNEL assay, we will quantify cellular death in response to carcinogen treatment. We hypothesize that *poli*-deficient mice will display lower levels of cell death after UV, measured partially by the appearance of apoptotic "sunburn cells," in correlation with their proposed increased cancer risk.

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APPENDIX

List of Abbreviations

7-AAD	7-aminoactinomycin D
AAAF	N-acetoxy-2-acetylaminofluorene
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia related
B[a]P	Benzo[a]pyrene
BPDE	7R,8S-dihydroxy-9S,10R-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene
BrdU	Bromodeoxyuridine
CPD	Cyclobutane pyrimidine dimer
DMEM	Dulbecco's modified Eagle's medium
DNA	Deoxyribonucleic acid
FACS	Fluorescence activated cell sorting
FBS	Fetal bovine serum
HPRT	Hypoxanthine phosphoribosyltransferase
IVT	In vitro transcription
miRNA	Micro-ribonucleic acid
mRNA	Messenger ribonucleic acid
NER	Nucleotide excision repair
NMSC	Non-melanoma skin cancer
NOP	1-nitrosopyrene
nt	nucleotide
PBS	Phosphate-buffered saline
PCNA	Proliferating cell nuclear antigen
pol	Polymerase

RAD	Radiation sensitive
REV	Reversionless
RPA	Replication protein A
TG	6-Thioguanine
TLS	Translesion synthesis
Trp53	Transformation related protein 53
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UV	Ultraviolet
XP	Xeroderma Pigmentosum

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PROFESSIONAL EXPERIENCE

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MEMBERSHIPS in PROFESSIONAL and SCIENTIFIC SOCIETIES

Organization	*Year*
Society of Undergraduate Chemistry Students, University of Louisville Vice President	2004 – 2006 2005 – 2006
Environmental Mutagen Society	2007 – Present
Ohio Valley Society of Toxicology	2007 – Present
American Association for Cancer Research	2008 – Present

HONORS

Title of award, sponsor	*Year*
Dean's List, Centre College	2002 – 2003
Dean's List, University of Louisville	2003 – 2006
Cancer Education Program Fellowship, University of Louisville	2005
First Place Poster, Research!Louisville	2007
Travel Award, Environmental Mutagen Society	2007
Travel Award, University of Louisville School of Medicine	2007
Travel Award, University of Louisville Graduate Student Council	2007

PROFESSIONAL ACTIVITIES

SERVICE:

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Chair Search Committee, Dept of Biochemisty & Molecular Biology 2007 – Present

MENTORING:

University of Louisville

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Christopher Belcher
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PRESENTATIONS:

Local / Regional

2005 Poster Presenter, Research Louisville, UofL
Chronic Inflammation Does Not Alter Patterns of Gene Expression in Carcinogen-Initiated Lung Cancer

2006 Poster Presenter, Posters-at-the-Capitol, Frankfort, KY
Chronic Inflammation Does Not Alter Patterns of Gene Expression in Carcinogen-Initiated Lung Cancer

2007 Seminar, Dept. of Pharmacology, UofL
Human DNA polymerase η is error-free when bypassing UV photoproducts but error-prone when bypassing benzo[a]pyrene adducts in vivo.

2007 Seminar, Student Meeting of the Ohio Valley Society of Toxicology, Louisville, KY
Human DNA polymerase η is error-free when bypassing UV photoproducts but error-prone when bypassing benzo[a]pyrene adducts in vivo.

2007 1st Place Poster, Basic Science Masters Student, Research!Louisville, UofL
DNA polymerase eta is the preferred translesion DNA polymerase and may be error-free or error-prone: evidence from mutation spectra.

2007 Poster Presenter, Ohio Valley Society of Toxicology Annual Meeting, Indianapolis, IN
Polη and Poli are Required for Mutagenic Bypass of BPDE Lesions in Primary Murine Fibroblasts

2007 Poster Presenter, JG Brown Cancer Center Annual Retreat, Louisville, KY
Polη and Poli are Required for Mutagenic Bypass of BPDE Lesions in Primary Murine Fibroblasts

National / International

2007 Platform Presentation, Environmental Mutagen Soc. National Meeting, Atlanta, GA
Evidence from Mutation Spectra that DNA Polymerase eta is the Preferred Translesion DNA Polymerase and May Be Error-Free or Error-Prone

2008 Platform Presentation, Environmental Mutagen Soc. National Meeting, Rio Grande, Puerto Rico
Cell Death and Cell Cycle Pathways as Potential Targets for Tumor Suppression by Pol Iota: A Systems Biology Approach

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RESEARCH

1. FUNDING

Current Grant Support:

Grant Source	Grant Title	Role in Project and % of Effort		Years Inclusive
NIEHS ES011564	U of L Environmental Health Sciences Training Program T32	T32 Fellow	100%	12/2007 – 11/2008

2. LIST of CURRENT RESEARCH INTERESTS:

a. Understand the cellular roles played by Y family polymerases in response to structurally varied DNA adducts
b. Utilize emerging nanotechnologies to develop targeted markers for imaging and treatment of skin and lung cancer.
c. Investigate the therapeutic effects of arsenic in mouse models of UV-induced skin cancer.

COMMUNITY INVOLVEMENT

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