Winter 12-2001

The effect of the human papillomavirus E5 protein on apoptosis in human foreskin keratinocytes

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A THESIS

Presented to the

Honors College at Southern University
Baton Rouge, Louisiana

In Partial Fulfillment of the Requirements for the
Honors College Degree

By

Kasey Pattan

December 2001

Honors College

Southern University and A& M College
Baton Rouge, Louisiana

CERTIFICATE OF APPROVAL

HONORS THESIS

This is to certify that the Honors Thesis of
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in Microbiology

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ACKNOWLEDGMENT OF RESEARCH

This research was supported by the Walter Cancer Institute School of Medicine at Indiana University Purdue University at Indianapolis, Indiana.

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ABSTRACT

There are over seventy genotypes of Human Papillomaviruses (HPVs), some cause benign warts and others are risk factors for the development of carcinomas of the respiratory and genital tract. Research has proven that the high risk HPV 16 E5 protein in the presence of epidermal growth factor (EGF) acts as a survival factor resulting in elevated levels of activated epidermal growth factor receptor (EGFR) and activated levels of ERK ½ and Akt, two signaling molecules downstream of EGFR, in UVB irradiated human foreskin keratinocytes (HFKs). This leads to the protection of the cells from UVB induced apoptosis. The purpose of this experiment is to determine if the anti-apoptotic activity of the E5 protein is unique to the development of cancer or if the E5 protein is critical to the completion of the life cycle of both the low and high risk viruses. The hypothesis being tested is that the anti-apoptotic activity of the E5 protein is fundamental to the completion of the life cycle of low and high risk HPVs. If this is correct then the low risk HPV 6 E5 protein will block apoptosis. Therefore, to test this hypothesis HFKs were infected with the retroviruses encoding HPV 16 E5, HPV 6 E5a, or the parent virus, LXSN. Then the cells were selcted with G418, an antibiotic, exposed to UVB light, fixed in ethanol and incubated with propidium Iodine (PI) and Rnase A. Fluorescence Activated Cell Sorter (FACS) analysis was performed. The presence of cells in the subG1 peak would indicate apoptosis. In addition, to determine the potential effect of low risk E5 on survival pathways, cells were lysed and Western blots were performed on the cell lysates to determine whether the EGFR had been activated

AUTHOR'S ACKNOWLEDGMENTS

The author, Kasey Lynette Pattan, would like to recognize the staff of Indiana University Purdue University at Indianapolis T35 and Summer Research Opportunity Program (SROP) for allowing her to participate in SROP 2001. Special recognition is given to the author's research advisor, Dr. Ann Roman, Ph.D. and the Microbiology/Immunology laboratory staff especially, Esra Smith and Benyue Zhang for assistance in the completion of her summer research project. The author would like to grant a special thanks to Dr. Oswald D'Vergne, Ph. D., MPH, for guidance in the completion of her thesis. Ms. Pattan would also like to thank the Southern University A&M College Honors College for support throughout her tenure at Southern University A&M College. Last but not least, Kasey Pattan would like to extend a special thanks to God and her family for making her college career a success.

TABLE OF CONTENTS

List of Figures

LIST OF APPENDIX

CHAPTER I
BACKGROUND

Introduction

The papillomaviruses are a group of small DNA viruses which induce warts or papillomas in a variety of higher vertebrates, including man. Historically, the papilloviruses were grouped together with the polymaviruses to form the papovavirus family. Papillomaviruses are small, nonenveloped, icosahedral DNA viruses that replicate in the nucleus of squamous epithelial cells (Toms, 1996). Seventy different papillomaviruses have been described. Since serologic reagents are not generally available to distinguish each of these types, they are not referred to as serotypes. The seventy geneotypes of human papillomaviruses (HPVs) cause benign warts and carcinomas of the epidermis, respiratory tract, and genital tract (Zhang, 2001). The HPVs can be referred to as high risk and low risk with respect to the development of cervical cancer in women. HPV 6 is an example of a low risk virus, and is the focus of this experiment, where as, HPV 16 is referred to as a high risk virus. HPV 16 is predominantly found in cervical carcinoma lesions, causing 5,000 deaths annually in the United States (Coffin, 1996). HPV 6 is predominantly found in genital wart lesions, the most prevalent viral sexually transmitted disease (Zhang, 2001). HPV 6 encodes two E5 proteins, E5a and E5b. HPV 6 E5a protein is a hydrophobic, leucine rich polypeptide with two helix breaking Pro residues at the amino and carboxy termini. It contains Cys-X-Cys residues near the carboxy terminus (toms, 1996). The E5a protein is known to transform immortalized human epidermal keratinocytes (Zhang, 2001).

Apoptosis, programmed cell death, is a physiological method of cell death that affects many aspects of natural life from embryonic development through cellular homeostasis and disease. During apoptosis cells undergo morphological and biochemical changes, which includes the loss of cell volume, internucleosomal DNA degradation, and the formation of apoptotic bodies (Schartz, 2001). Ultraviolet radiation is a stress factor that promotes cell death in human foreskin keratinocytes (HFKs) (Szabo, 1995). Ultraviolet radiation can be divided into three parts by wavelength: UVA (320-400 nm), UVB (280-320 nm), and UVC (200-280 nm). The apoptotic device in this experiment was UVB.

Flow cytometry is a fast and easy way to determine changes in cell size during apoptosis. Variations in the physical characteristics of a cell are assessed with flow cytometry by examining changes in its light-scattering properties. A change in the light-scattering property of a cell in the forward direction is a direct measure of cell size (Campbell, 1991). When a cell undergoes apoptosis, the cell shrinks, with an observed decrease in forward scattered light (Benjamin, 2000). Fluorescent Activated Cell Sorting (FACS) of DNA content is used in cell cycle research because cells in different phases of the cell cycle have different amounts of DNA. G0/G1, G2/M and S phase have a 2, 4, and intermediate DNA content, respectively. This is extremely helpful in studying apoptotic cells. DNA intercalating dyes, such as propidium iodide, fluoresce with an intensity proportional to bound DNA, making it relatively easy to estimate the DNA content cell cycle distribution by using flow cytometry (Benjamin, 2000). The two main FACS

instruments are Becton Dickinson, which was used for this experiment, and Coulter Electronics.

A Western blot, in common usage, refers to the electrophoresis of the antigen on SDS-PAGE gel followed by its subsequent transfer to nitrocellulose. The paper is then incubated with a specific antibody followed by a labeled polyclonal secondary antibody (Campbell, 1991). Western blotting was developed by Towbin with polyclonal antibodies as a tool for hybridoma technology (Toms, 1996). All the Western blot techniques involve the transfer of elctrophoretically separated material transferred to nitrocellulose paper, where the various bands can be assessed for binding to a probe which is labeled with an enzyme, isotope, or flurophor (Campbell, 1991). Hybridoma technology allows the production of monoclonal antibodies. Polyclonal preparations result from the response to immunization or recovery from infection in a host. Polyclonal preparations contain antibodies to multiple antigens and includes antibodies of various isotopes. Monoclonal has one specificity and one isotope (Benjamin, 2000).

Ratinale for Research

The purpose of this experiment is to determine if the anti-apoptotic activity of the HPV E5 protein is unique to the development of cancer, and therefore, only seen in the high risk viruses or if the E5 protein is critical to the completion of the life cycle of low and high risk HPVs. This will be determined using two assays, FACS analysis and Western blot.

Proposed Results of Research

The hypothesis being tested is that the anti-apoptotic activity of the E5 protein is critical to the completion of the life cycle of the low and high risk HPVs. FACS analysis results will indicate that LXSN infected cells are more apoptotic than HPV 16 E5 and HPV 6E5a infected cells. The Western blot results will indicate that there is more phosphorylation in HPV 6E5a and HPV 6E5b protein lysates than in LXSN protein lysates.

CHAPTER II
REVIEW OF LITERATURE

Dangers of Human Papillomaviruses

Cervical carcinoma is among the most common cancer related causes of death among men and women worldwide. Infection with human papillomavirus, a small DNA tumor virus, plays a role in the malignant transformation of cervical epithelium. Of the genital HPVs , HPV type 6 and HPV 11 are the most common isolates and are associated primarily with benign lesions as condyloma acuminata, a portion of the premalignanant cervical intraepithelial neoplasias. HPV 16 and HPV 18 are associated with 60 to 90% of cervical intraepithelial neoplasias and 90% of malignant disease. Thus leading into a correlation between infection with these HPV types and progression to invasive cervical cancer (Straight, 1993).

HPVs are known to induce epithelial cell proliferation during a productive infection. In the United States, an estimated one million cases of genital warts occur annually as a result of HPV 6. HPV 6 also causes juvenile and adult respiratory tract papillomatosis. Respiratory papillomata are noninvasive proliferative lesions of the respiratory epithelium. The incidence of recurrent respiratory papillomatosis has been estimated at 1,500 cases with more than 50% of the cases occurring in children under the age of three (Chen, 1990).

Previous Research on Human Papillomaviruses

Faulkner and Banks research on HPVs demonstrated that HPV 16 E5 could stimulate proliferation of primary baby rat kidney (BRK) cells in the presence of HPV 16 E7. Their research also showed that cooperation between the HPV 16 E7 and E5 genes can result in an increased proliferative capacity in primary BRK cells which is enhanced in the presence of EGF. Upon experimentation the scientists concluded that the co-mitogenic activity conserved between benign and oncogenic associated HPV types represents a conserved function that is related more to viral replication than cell transformation (Faulker, 1995).

Crusis reported that cells expressing the HPV 16 E5 open reading frame showed a greatly enhanced transcription of the immediate early genes after EGF treatment when compared to control cells. This enhancement is due to amplification of the signal transduction pathways in response to the growth factors. Upon short time EGF treatment of the E5 expressing cells the scientists observed an increase in the activation of EGF receptors, resulting in a stronger activation of MAP kinase and ERK ½ when compared to control transfected cells (Crusius, 1997).

Chen and researchers investigated the mechanism by which HPV stimulates cell proliferation during infection. They reported that the E5a gene of HPV 6 is capable of transforming NIH 3T3 cells. Their experimentation demonstrated that the translation of the E5a open reading frame is required for biological activity. This was the first demonstration of a transforming oncogene in HPV 6 (Chen, 1990).

Gu and scientists investigated the effect of the oncogenic HPV 16 E5, E6, and E7 genes on the induction of the MAP kinase signaling pathway. The E5 gene induced an increase in the MAP kinase activity both in the presence and absence of EGF. These findings suggest that E5 may function, at least in part, to enhance the cell response through the MAP kinase pathway. These findings are also consistent with E5 enhancing the response to growth factor stimulation (GU, 1995).

CHAPTER III
METHODOLOGY

FACS Analysis

Remove human foreskin keratinocytes of cell types F551 or F554 from a nitrogen storage tank. Wear thermal gloves to protect the hands while removing the cells from the nitrogen tank. Place the cells on dry ice while transporting them to a 37° C water bath. Incubate the cells in the water bath until they thaw. While the cells are thawing make a complete keratinocyte serum free media (CK-SFM) with 50 mg/ml of Genamycin, an antibiotic that prevents the media from becoming contaminated. Place the cells in three coning tubes with 6ml of CK-SFM under a fume hood. Centrifuge the cells at 800 rmp for five minutes. While centrifuging the cells label four petri dishes with the cell type F551 or F554. Remove the cells from the centrifuge and aspirate the CK-SFM from the cells under the fume hood. Mix the cells with 32 ml of CK-SFM using a pipette. Pipette 8 ml of the mixture containing the cells into each petri dish. Incubate the cells overnight at 37°C.

The following day remove the LXSN, HPV 16E5 and HPV 6E5a viruses from a -70°C freezer using protective thermal gloves. Thaw the viruses in a 37°C water bath. While the viruses are thawing remove the cells from the incubator and aspirate the media from the petri dishes under the hood. Label three of the four petri dishes with the above mentioned viruses. Infect the cells with the appropriate labeled virus by pipetting 5 ml of LXSN and HPV 16E5 with 5ml of DMEM (cell media used to dilute the high titer virus) on the appropriate labeled petri dish. Pipette 10 ml of HPV 6E5a on the appropriate labeled petri dish. (Ten milliliters of HPV 6E5a is used for infection because the virus

has a lower titer than LXSN and HPV 16E5.) Add 26 ul of polybrene to each petri dish to aid the virus entrance into the cells. Incubate the cells at 37°C for six total hours. The fourth petri dish of cells is also incubated in case an infection error occurs or if any of the cells die. Reinfect the low titer HPV 6E5a infected cells after three hours. After three additional hours aspirate the viruses from each petri dish. Wash the cells with 3 ml of CK-SFM. Aspirate the petri dishes and then add 8 ml of CK-SFM to each petri dish. Incubate at 37°C for two days.

Warm a bottle of trypsin, fetal bovine serum, and CK-SFM in a 37°C water bath. While the media is warming label three coning tubes with the following viruses: LXSN, HPV 16E5, and HPV 6 E5a. Add 2 ml of fetal bovine serum to each coning tube under the fume hood. (Fetal bovine serum stops the cells from trypsinizing.) Aspirate the media from the petri dishes. Add 1 ml of trypsin to each petri dish to remove the cells from the dish. Incubate the petri dishes at 37°C for two minutes. Using a shortie pipette wash the cells down the petri dish in a clockwise direction. Place the cells in the appropriate labeled coning tube. Rewash the plate with the media from each coning tube to ensure that all of the cells are removed. Centrifuge the cells at 1550 rpm for five minutes. While centrifuging the cells label ten petri dishes, which will be used once the cells are split. Label four petri dishes for LXSN and HPV 16 E5, which will be split on a 1:4 ratio. Label two dishes with HPV 6E5a, which will be split on a 1:2 ratio. Mix each coning tube with a pipette and place 8 ml of the mixture onto the appropriate labeled petri dish. Place the petri dishes into a 37°C incubator for three hours. After three hours add

21 ml of G418, an antibiotic used for selection, to each petri dish. The G418 antibiotic kills the cells that were not infected with the viruses. Only the infected cells can be used for experimentation. Incubate at 37°C for three days.

Warm the CK-SFM in a 37°C water bath. Remove the dishes from the incubator. View the dishes under the microscope. Very few cells should be visible because the cells were selected with the G418 antibiotic. Aspirate the media from the plates. Feed the cells with 8 ml of CK-SFM, which is added to each petri dish. Incubate the cells at 37°C for two days.

Warm the trypsin, fetal bovine serum, and CK-SFM in a 37°C water bath. While the media is warming label three coning tubes with the three virus types, LXSN, HPV 16 E5, and HPV 6E5a. Place 2 ml of fetal bovine serum in each coning tube. Add 1 ml of trypsin to each petri dish. Incubate each dish for two minutes at 37°C. Wash the cells down each petri dish with the trypsin and transfer the cells to the labeled coning tubes. Centrifuge the cells at 1550 rpm for five minutes. Aspirate the media off the cells in the coning tubes. Add 10 ml of CK-SFM to each coning tube. Mix the solution with a pipette. Pipette 15 ul of the solution from each coning tube. Place the 15 ul of the solution in a hemacytometer, a cell counter. Count the total number of cells under the microscope. Determine the configuration to get an even distribution of the total number of cells so that each of the seven total petri dishes will have the same number of cells. Place 56 ml of CK-SFM into each coning tube and pipette 8 ml of the solution unto each one of the petri dishes. Incubate the cells at 37°C overnight.

Warm the UVB box under the hood. Warm the CK-SFM and PBS (a saline solution) in a 37°C water bath. Aspirate the media off the infected cells. Place 8 ml of PBS in each petri dish. Expose each group of infected cells to UVB for 0J, 200J, 400J, and 600J. Aspirate the media from each petri dish and add 8 ml of CK-SFM to each petri dish. Incubate at 37°C overnight.

Warm the fetal bovine serum and trypsin in a 37°C water bath. Label twelve coning tubes with the virus type, (LXSN, HPV 16E5, or HPV 6E5a), and UVB exposure, (0J, 200J, 400J, and 600J). Add 1 ml of fetal bovine serum to each coning tube. Add 1 ml of trypsin to each petri dish. Incubate the petri dishes at 37°C for 8 minutes. Remove the petri dishes from the incubator and wash the cells down the plate with trypsin. Place the cells in the appropriate labeled coning tube. Centrifuge at 1550 rpm for 5 minutes. Aspirate the media from each coning tube. Add 300 ul of PBS to each coning tube and mix with a micropipette. Hold the solution in the micropipette and add 5 ml of cold ethanol to each coning tube. Inject the mixture contained in the micropipette into the cold ethanol. (The cold methanol helps to maintain the cells morphology and it prevents the cells from clumping together.) Refrigerate overnight.

Remove the cells from the refrigerator. Centrifuge at 1550 rpm for 5 minutes. Aspirate the media from each coning tube. Add 5 ml of PBS to each coning tube. Vortex each coning tube to ensure that the cells are mixed and separated. Let the cells stand for 5 minutes. Centrifuge the cells again at 1550 rpm for 5 minutes. Stain the cells with 1 ml of propidium iodine and digest the cells RNA by adding Rnase A to each coning tube.

Place the coning tubes in a test tube rack and store in a sealed box to keep the light out. Transport the cells to the FACS laboratory. Vortex each coning tube and pipette 1 ml of each solution into the FACS test tubes. Vortex each FACS test tube and perform FACS analysis on each test tube. Save the FACS results for interpretation and presentation using a computer program called Cell Quest.

Western Blot

Wash the Western blot apparatus glass plates and gaskets. Slide one of the glass plates into the gasket. Place one spacer on the right and left side of the glass. Cover the glass with the companion glass piece. Place two clamps at the bottom of the glasses and two clamps on the right and left side of the glasses. Make a 10% SDS-PAGE gel. To make the separating portion of the gel place 4 ml of water into a 40 ml beaker. Add 3.3 ml of 33% acrlymide mix, 2.5 ml of 1.5 M Tris, 0.1 ml of 10% SDS, 0.1 ml of APS, and .0004 ml of TEMED to the beaker. Pipette the mixture into the apparatus. Add water across the top of the mixture in the apparatus. Let stand for thirty minutes so that the gel will solidify. While the gel is solidifying make the stacking gel. Place 2.3 ml of water in a 40 ml glass beaker. Add 1.3 ml of .5M Tris, 0.9 ml of 30% acrylmide, and 50 ul of 10% SDS. Pour the water off the glass apparatus. Dry the inside of the glass apparatus. The inside of the apparatus is dried thoroughly because APS polymerizes very quickly in the presence of water. Add 50 ul of APS and 4 ul of TEMED to the solution in the glass beaker. Quickly add the solution to the apparatus. Place a comb between the glasses. Let the gel stand for one hour. Place running buffer in an electrophoresis buffer chamber.

Remove the nitrocellulose from the transfer cell. Place the nitrocellulose in a staining glass. Shake the nitrocellulose while washing it with TBST, a detergent. Pour off the TBST. Add 10 ml of panceaus red to stain the protein on the nitrocellulose. Shake for 15 minutes. Pour off the panceaus red and destain with acetic acid. Block the nitrocellulose with 5% milk. Shake for two hours. Dilute the GAPDH primary antibody by adding 1 ul of GAPDH to 20 ml of 5% milk. Pour off the milk and place the primary antibody on the nitrocellulose. Shake for one hour. Wash the nitrocellulose four times with 20 ml of TBST while shaking for ten minutes each time. Pour off the TBST. Dilute the secondary antibody by combining 1 ul of rabbit anti-mouse HRP (horseradish peroxidase) to 10 ml of milk. Place the secondary antibody on the nitrocellulose and shake for one hour. Wash the nitrocellulose five times with 20 ml of TBST while shaking every ten minutes.

Tear a small piece of saran paper. Add 2 ml of ECL (enhanced chemiluminescence) 1 and ECL 2 (the illuminated detection system) to a coning tube. Remove the nitrocellulose from the shaker using forceps and place in a staining glass. Place the ECL 1 and ECL 2 system on the nitrocellulose for one minute. Remove the nitrocellulose with forceps and dry the edges on a piece of filter paper. Place the nitrocellulose on the saran wrap and cover completely, making sure that the saran coverage is completely smooth. Place the nitrocellulose in an autoradiography cassette. Gather x-ray film and proceed to a dark room. Expose the nitrocellulose to x-ray film for 10 seconds, 1minute, and 5 minutes.

To strip the nitrocellulose add 50 ml of stripping buffer to a staining glass. Add 350 ul of 2-mercapto-ethanol (ME) to the stripping buffer under the hood. Top the glass and then cover with saran wrap to keep the pungent odor concealed. Shake in a 65°C water bath for 30 minutes. Transfer the nitrocellulose to a smaller concentration glass and wash three times with 20 ml of TBST while shaking for ten minutes. Pour off the TBST and add 15 ml of 5% milk and shake for one hour. Wash two times with 20 ml of TBST while shaking for five minutes. Pour off the TBST. Add 1 ml of TYR 845 Phospho-EGF Receptor primary antibody to the nitrocellulose. Shake overnight at 4°C. Pipette the antibody off the nitrocellulose and store in a coning tube. Wash four times with 20 ml of TBST while shaking each time for ten minutes. Add 1.3 ul of the secondary antibody, goat anti-rabbit, to 10 ml of 5% milk in a coning tube. Pour off the TBST. Incubate the nitrocellulose in the secondary antibody while shaking for one hour. Pour off the secondary antibody and wash five times with 20 ml of TBST while shaking for ten minutes each time. Place 2 ml of ECL 1 and ECL 2 in a coning tube. Cut a small piece of saran wrap. Remove the nitrocellulose from the dish using forceps and dry the edges on a piece of filter paper. Place the nitrocellulose in another staining glass and incubate in the ECL system for 2 minutes. (Two minutes were used because the results from the 1 minute exposure to the ECL system were very vague.) Remove the nitrocellulose, dry the edges and wrap in the saran paper. Proceed to the dark room and expose the nitrocellulose in an autoradiography cassette using x-ray film for 10 seconds, 30 seconds, 1 minute, and 5 minutes. Repeat the entire procedure from the initial step of

CHAPTER IV
RESULTS

FACS Analysis

Figure 1 is a FACS generated simulation of the LXSN, HPV 16E5, and HPV 6E5a infected cells that were exposed to UVB. The section of concentration on each diagram is M1. This portion of each diagram represents the cells in the G0 stage. During this stage the cells undergo apoptosis. The cells infected with LXSN, HPV 16 E5, and HPV 6E5a exposed to 0 J of UVB underwent less apoptosis than the infected cells exposed to 400 J of UVB. The LXSN infected cells underwent more apoptosis at exposure 400 J of UVB than the HPV 16 E5 and HPV 6 E5a infected cells.

Figure 2 is a bar graph representation of the cells that underwent apoptosis at different UVB exposures. All of the cells experienced very little apoptotic activity when exposed to 0 J of UVB. At 200 J of UVB exposure the LXSN infected cells experienced the least amount of apoptotic activity and the HPV 16 E5 infected cells experienced the most apoptosis. This was inconsistent with the hypothesis. At 400 J the LXSN infected cells underwent the most apoptosis and the HPV 16E5 infected cells underwent the least amount of apoptosis. At 600 J of UVB exposure the LXSN infected cells were the most apoptotic and the HPV 16 E5 infected cells were the least apoptotic. Both of these results were consistent with the hypothesis.

Figure 3 is another bar graph representation of the cells that underwent apoptosis At different UVB exposures. There was very little apoptotic activity at 0 J UVB exposure in

all of the cells. At 200 J the LXSN infected cells experienced the most apoptotic activity and the HPV 6E5a infected cells experienced the least apoptotic activity. At 400J UVB exposure the LXSN infected cells underwent the most apoptosis and the HPV 6E5a infected cells underwent the least amount of apoptosis. At 600J the LXSN infected cells were the most apoptotic and no results were obtained from the HPV 6E5a infected cells.

Western Blot

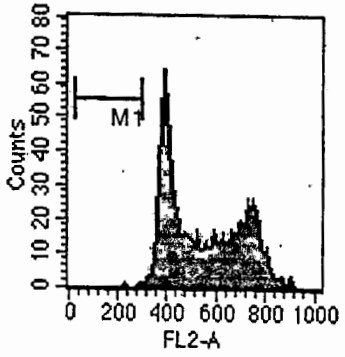
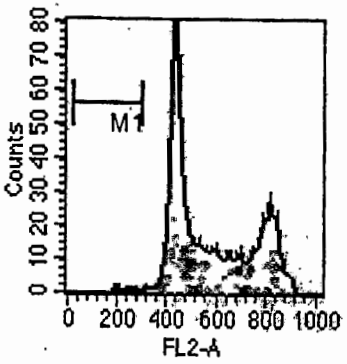
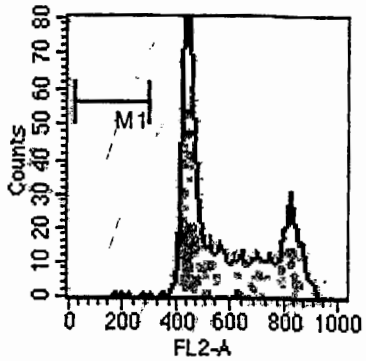
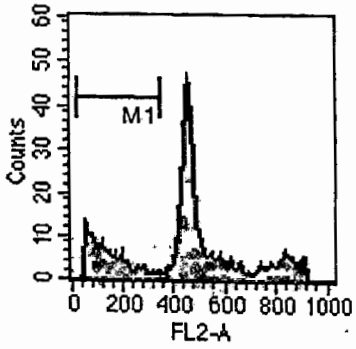
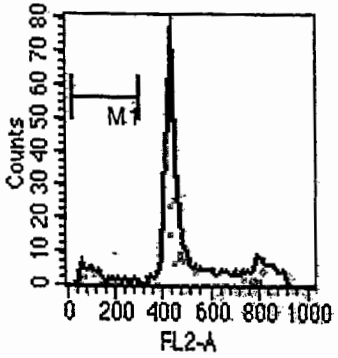
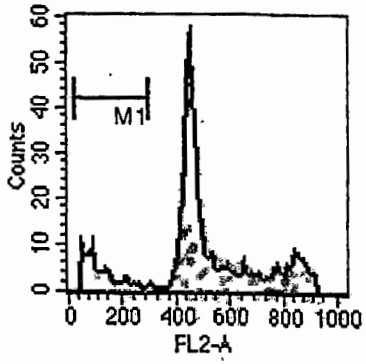
Figure 4 is the results from the Western blot using a densitometric computer program. The proteins tested were lysates from LXSN, HPV 6 E5a, and HPV 6 E5b. Each of the cells were starved for EGF for 24 hours, then stimulated with EGF and lysed after 0 minutes, 15 minutes, 30 minutes, and 300 minutes. These lysates were loaded into the Western blot SDS Page and analyzed using autoradiography. The three antibodies used in this assay were Phospho-EGFR, EGFR, and GAPDH (as the control). All of the infected cells experienced very little phosphorylation after 0 minutes of stimulation with EGF. After 15 minutes of EGF stimulation the LXSN lysates experienced the least amount of phosphorylation and the HPV 6E5a lystes experienced the greater amount of phosphylation. After 30 minutes of EGF stimulation the HPV 6E5a lysates experienced the most phosphorlaytion and the HPV 6E5b lysates phosphorlated the least. This was very inconsistent with the hypothesis. After 300 minutes of stimulation with the EGF the LXSN lysates phosphorylated the most and the HPV 6E5b lystaes phosphorylated the least. This was another inconsistency with the hypothesis.

CHAPTER V
CONCLUSION

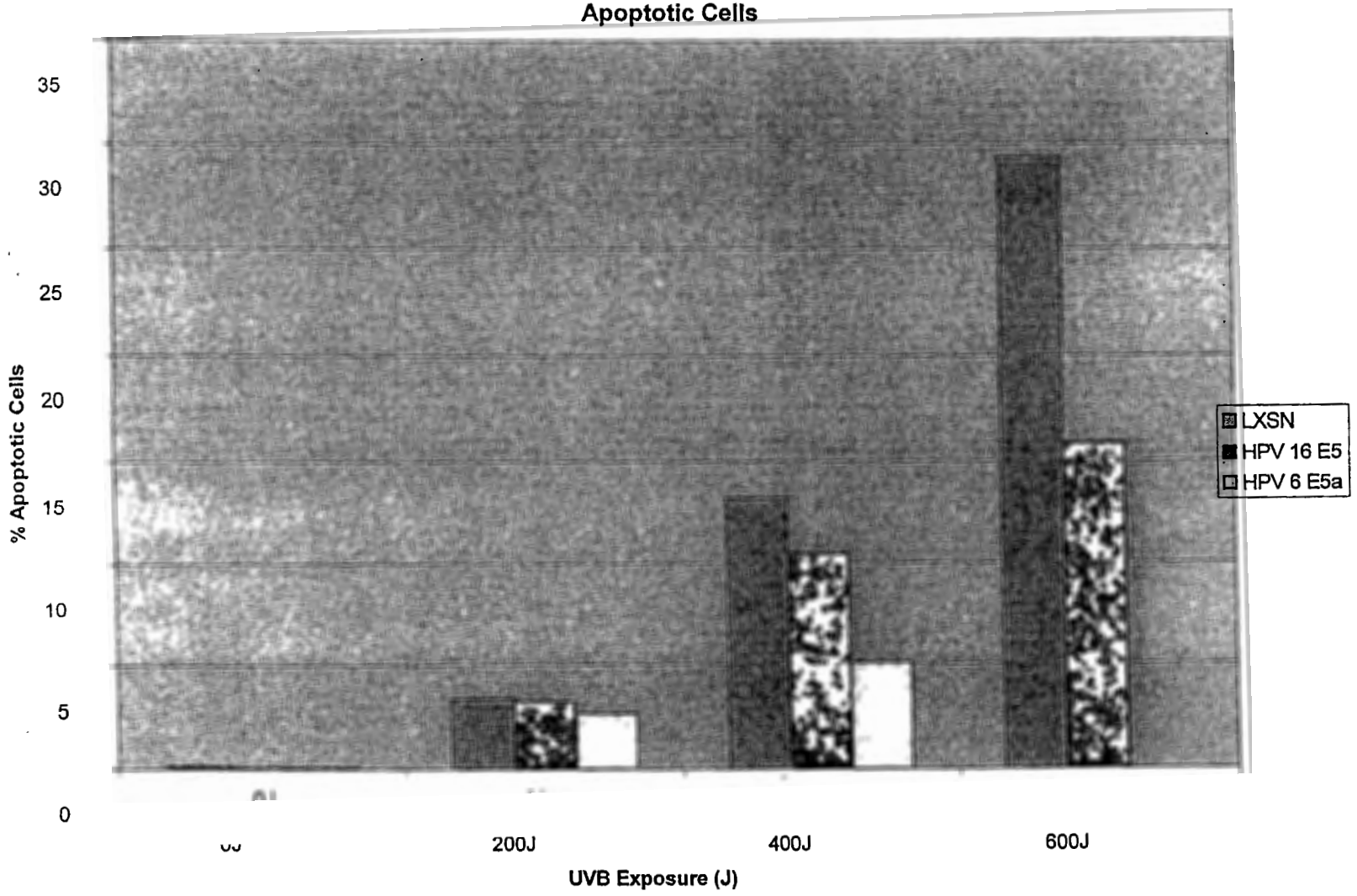
The results from Figure 1 indicate that LXSN infected cells undergo more apoptosis than HPV 16E5 and HPV 6E5a infected cells. According to Figure 2 LXSN infected cells also experience more apoptotic activity than HPV 16 E5 and HPV 6E5a infected cells. However, there was a discrepancy at 200 J when the LXSN infected cells were the least apoptotic. All of the results in figure 3 indicate that LXSN infected cells undergo more apoptotic activity than HPV 16E5 and HPV 6E5A infected cells. Figure 4 indicates that after 15 minutes of EGF stimulation the HPV 6E5a lysates were the most phosphorylated and the LXSN lysates were the least phosphorylated. After 30 minutes of EGF stimulation the HPV 6E5a lysates were again the most stimulated; however, the HPV 6 E5b lysates were the least phosphorylated. This was a discrepancy. After 300 minutes the greatest discrepancy occurred when the LXSN lysates were the most phosphorylated.

No conclusion can be made from the preliminary results obtained from the FACS analysis or Western blot to determine whether or not HPV 6E5a protein prevents apoptosis in human foreskin keratinocytes. From the FACS analysis most of the results indicate that LXSN infected human foreskin keratinocytes underwent more apoptotic activity than HPV 6E5a infected human foreskin keratinocytes. The discrepancy at 200J may have been due to the fact he 200 J of UVB exposure is too low of a dosage to give accurate results. Therefore, the results from all the FACS analysis were not consistent.

FACS Analysis Results (Figure 1)

	LXSN	16 E5	6 E5a
0 J/m^2			
400 J/m^2			

FACS Analysis Results (Figure 3)



It is believed that if HPV 6E5a enhances the activation of EGFRs than it will also elevate the survival pathways, ERK ½ and Akt. This will increase the phosphorylation of the EGF stimulated lysates and thus prevent the cells from undergoing apoptosis. However, the results from the Western blot were too inconsistent. There was never enough consistency in the amount of phosphorylation to determine which lysates were more phosphorylated. The lysates of focus were the 15 and 30 minute EGF stimulated lysates because after 300 minutes the phosphorylation of the lysates decreases due to over stimulation. There was no consistency in these two time frames.

This experiment can be improved with more precision. For example, the time and the weather conditions of day that the FACS analysis was performed should be consistent. The amount of sunlight may play a role in the reading acquired from the Becton Dickinson FACS machine. Other assayes should be used to measure apoptosis and more than 6 weeks of research is needed to obtain the precise protocols to develop an answer to such an intense question.

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VITA

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Kasey is a native of Port Allen, Louisiana. She is the daughter of Mr. and Mrs. Gordie A. Pattan III. Kasey has an older sister, younger brother, three nephews, and a host of aunts, uncles, and cousins. Miss Pattan was reared by her grandmother, Mrs. Joyce W. Pattan. God is the head of Kasey's life as she is a very active member of Scott's United Methodist Church. Kasey's motto in life is, 'My life is in His Hands'. She thanks God and her family and friends for all that she has accomplished in life.

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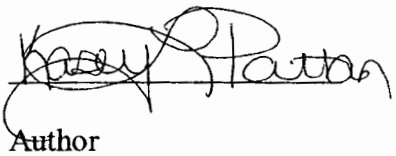

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