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13. ABSTRACT *(Maximum 200 Words)*

The proposed research project investigates the relationship between exposure to cigarette smoke and genetic susceptibility factors. Levels of 4-aminobiphenyl (4-ABP)-DNA adducts in breast tumor tissue sections were the same between smokers and non-smokers. The project will also investigate whether an association exists between 4-ABP-DNA adducts and the carcinogen metabolizing genes CYP1A2 and NAT2. The analysis of the CYP1A2 gene is currently in progress. Due to the difficulty in obtaining large fragments of DNA from the tumor tissue sections required for PCR-RFLP, a new method is under development for genotyping NAT2. Another aim of this project is to determine if environmental chemicals and their metabolites can cause mutations in the p53 gene. We hope to analyze the mutations in p53 with the innovative Affymetrix system recently purchased by Columbia University. After the completion of advanced coursework in statistics and epidemiology this fall, the correlations made between adduct levels and genotyping data of the metabolizing enzymes will be available for next year's report.

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Table of Contents

Introduction

The proposed research project investigates the relationship between exposure to cigarette smoke and genetic susceptibility factors. Levels of 4-aminobiphenyl (4-ABP)-DNA adducts in breast tumor tissue sections were not significantly different between smokers and non-smokers. The project will also investigate whether an association exists between 4-ABP-DNA adducts and the carcinogen metabolizing genes CYP1A2 and NAT2. The analysis of the CYP1A2 gene is currently in progress. Due to difficulty in obtaining large fragments of DNA from the tumor tissue sections, a more sensitive and specific method is under development for genotyping NAT2. Another aim of this project is to determine if environmental chemicals and their metabolites can cause mutations in the p53 gene. We hope to analyze the mutations in p53 with the innovative Affymetrix system currently purchased by Columbia University.

4-ABP-DNA Adducts

The immunohistochemical analysis of the 4-ABP-DNA adducts in breast tissue was completed this year. Difficulties arose in acquiring a difference in staining between the positive and negative controls. The control samples consist of 4-ABP treated and untreated cultured R52 cells that contain CYP1A2 enzymatic activity. This has been proven to be an effective method for this laboratory and other immunohistochemical techniques. The untreated and treated cells did have a significant difference in staining intensity, but once they were embedded into paraffin blocks and sectioned onto slides, they lost their difference. Varying a number of steps in the immunohistochemistry method in hopes to obtain consistent and discernable differences for the controls was exhaustive. We have settled on using a low and a high staining liver tumor section as quality control samples. The quality control results from the ten batches of 15 samples are listed in Table 1.

Table 1 provides the preliminary results.. At this time, no apparent differences in 4-ABP-DNA adducts in breast tumor tissue exist between smokers and non-smokers. Intensity of smoking habits, such as cigarettes per day, years smoked, and age at initiation will be used in a more comprehensive analysis. Once I enhance my training in statistics this fall

with the Applied Regression course and an Epidemiology III lab, I will be able to provide a more detailed analysis of the staining data

CYP1A2

At the time of the proposal no phenotypic data existed for the polymorphisms found for CYP1A2. An inclusive summary of the current publications can be found in Table 2. Only two polymorphisms have been found to have phenotypic changes in enzymatic activity. The genotyping of CYP1A2*1C and *1F are currently underway and will be completed by the end of this month. The correlation between polymorphisms and adduct level will be completed by the end of this academic term when I complete my training in statistics.

NAT2

The three polymorphisms(NAT2*5, *6, *7) identified by the proposed PCR-RFLP technique, deemed to difficult due to the fragility of DNA obtained from the tumor sections. The established method required the amplification of a large 1200 base pair piece of DNA. The isolation of DNA from paraffin tumor tissue sections involved deparaffinization and the scraping of the section off of the microscope slide. The DNA was isolated with a phenol:chloroform extraction. The ability to amplify a large piece of DNA is unfeasible. This past summer we have developed a new technique for detecting single nucleotide polymorphisms that only requires the amplification of a short oligonucleotide (20-30bp). The Fluorescence Polarization-dye-terminator incorporation assay (FP-TDI) employs a primer that ends one base immediately 3' from the polymorphic site.(Chen 1999) In the presence of DNA polymerase and the allele-specific dye-labelled dideoxynucleoside triphosphate(ddNTP) the alleles present in the target DNA can be inferred. This method will prove to be beneficial to this proposal, not only for it's efficiency and accuracy, but also for the ability to analyze smaller, fragmented DNA samples.

Mutational Spectra of p53

Preliminary PCR amplification of exon 5-9 for several samples have been completed and were successful. We proposed to analyze the 150 samples by the conventional technique of PCR-SSCP. With the advent of Columbia University's purchase of the Affymetrix system and our lab's collaboration with Ainsley Weston of CDC we intend to analyze the 150 samples with Affymetrix Gene Chips. The analysis of the entire coding region of the human p53 tumor suppressor gene (exons 2-11) identifies missense mutations and single base deletions. This method has been shown to have 94% accuracy, 92% sensitivity and 100% specificity when compared to the conventional PCR-SSCP method.(Wen 2000)

Key Research Accomplishments

Task 1. (Months 1-6) Complete
Detect 4-ABP-DNA adducts in breast tissue using immunohistochemistry.

Task 2. (Months 7-13) Altered, in progress
Genotype the metabolizing enzyme CYP1A2 and NAT2.

Task 3. (Months 14-27) Future work
Determine p53 mutational spectra.

Task 4. (Months 28-32) Future Work
Correlations.
-Relationship of 4-ABP-DNA adducts to NAT2 and CYP1A2 genotypes
-Relationship of 4-ABP-DNA adducts and breast cancer
-Relationship of p53 mutations and expression to breast cancer

Task 5. (Months 33-36) Future Work
Write dissertation.

Reportable Outcomes

The only data available is the 4-ABP-DNA adduct levels found in breast tumor tissue. As summarized in Table 1, the levels are the same between smokers and non-smokers.

Conclusions

While the first year proved to be slow in obtaining reportable data, there was success in acquiring skills in more innovative approaches to DNA analysis. These methods will be employed to give very specific and sensitive results for next year's report. The advanced coursework in statistics and epidemiology will be employed while correlating adduct levels with the genotyping data. Utilizing the Affymetrix system for mutational analysis will not only be more efficient, but provide me with the opportunity to acquire new skills in Dr. Santella's laboratory.

References

Chen X, Levine L, Kwok PW. Fluorescence Polarization in Homogenous Nucleic Acid Analysis.492-498. Genome Research. Cold Spring Harbor Laboratory Press. 1999.

Chida M, Yokoi T, Fukui T, Kinoshita M, Yokota J, Kamataki T. Detection of three genetic polymorphisms in the 5'-flanking region and intron 1 of human CYP1A2 in the Japanese population. *Jpn J Cancer Res* 1999 Sep;90(9):899-902

Huang JD, Guo WC, Lai MD, Guo YL, Lambert GH. Detection of a novel cytochrome P-450 1A2 polymorphism (F21L) in Chinese. *Drug Metab Dispos* 1999 Jan;27(1):98-101.

Ikeya K, Jaiswal AK, Owens RA, Jones JE, Nebert DW, Kimura S. Human CYP1A2: sequence, gene structure, comparison with the mouse and rat orthologous gene, and differences in liver 1A2 mRNA expression. *Mol Endocrinol* 1989 Sep;3(9):1399-408.

Nakajima M, Yokoi T, Mizutani M, Shin S, Kadlubar FF, Kamataki T. Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the CYP1A2 gene. *Cancer Epidemiol Biomarkers Prev* 1994 Jul-Aug;3(5):413-21.

Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T. Genetic polymorphism in the 5'-flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. *J Biochem* (Tokyo) 1999 Apr;125(4):803-8.

Quattrochi LC, Tukey RH. The human cytochrome Cyp1A2 gene contains regulatory elements responsive to 3-methylcholanthrene. *Mol Pharmacol* 1989 Jul;36(1):66-71.

Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C-->A polymorphism in intron 1 of thecytochrome P450 CYP1A2 gene tested with caffeine. *Br J Clin Pharmacol* 1999 Apr;47(4):445-9

Welfare MR, Aitkin M, Bassendine MF, Daly AK. Detailed modelling of caffeine metabolism and examination of the CYP1A2 gene: lack of a polymorphism in CYP1A2 in Caucasians. *Pharmacogenetics* 1999 Jun;9(3):367-75.

Wen WH, Bernstein L, Lescallet J, Beazer-Barclay Y, Sullivan-Halley J, White M, Press MF. Comparison of TP53 Mutations Identified by oligonucleotide microarray and conventional DNA sequence analysis. *Cancer Research* 2000 May;60:2716-2722.

Table 1

		4-ABP-DNA staining intensity	
		average	stdev
controls	n		
positive	10	0.629	0.060
negative	10	0.330	0.038
smoker	63	0.314	0.173
non-smoker	74	0.314	0.165

Table 2

http://www.imm.ki.se/CYPalleles/cyp1a2.htm

CYP1A2 allele nomenclature

Allele	*Protein*	*Nucleotide changes*	*Trivial name*	*Effect*	*Enzyme activity*		*References*
					In vivo	*In vitro*	
*CYP1A2*1A*	CYP1A2.1	None	*Wild-type*		Normal	Normal	Ikeya *et al*, 1989 Quattrochi and Tukey, 1989
*CYP1A2*1B*	CYP1A2.1	5381T>C					Nakajima *et al*, 1994 Welfare *et al*, 1999
*CYP1A2*1C*	CYP1A2.1	-3858G>A			Decreased		Nakajima *et al*, 1999
*CYP1A2*1D*	CYP1A2.1	-2464delT					Japanese patent number 05719026 Chida *et al*, 1999
*CYP1A2*1E*	CYP1A2.1	-740T>G					Japanese patent number 05719026 Chida *et al*, 1999
*CYP1A2*1F*	CYP1A2.1	-164C>A			Higher inducibility		Japanese patent number 05719026 Sachse *et al*, 1999 Chida *et al*, 1999
*CYP1A2*2*	CYP1A2.2	63C>G		F21L	?	?	Huang *et al*, 1999