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TITLE: Polycyclic Aromatic Hydrocarbon (PAH)-DNA Adducts and Breast Cancer

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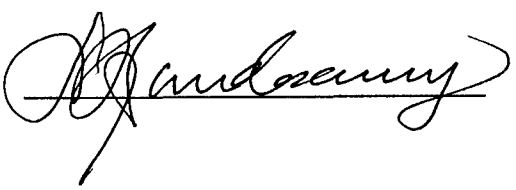

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13. ABSTRACT *(Maximum 200 Words)*

While immunofluoresence has begun on the 800 samples proposed,the start date for this was pushed back from the original date proposed to 6/99 due to equipment difficulties and the purchase of a new camera and software to increase sensitivity of the assay. In the meantime,the principal investigator, Sharon Hayes began and finished a project not originally proposed in the funded grant proposal. The 800 breast cancer cases and controls have been genotyped for a polymorphism in the DNA repair gene XPD. (Cancer Research 58, 604-608, Feb 15,1998) The samples are from the parent study, the Long Island Breast Cancer Research Project, and we are not permitted to publish this data until the primary hypotheses from this study are published. I am blinded to the status of the samples until I finish the immunfluoresence then both papers should be published. The immunofluorescence should be finished within 6 months and analysis of both the 800 samples for both projects (originally funded and the XPD project) should begin at that time. We have received 60 tissue samples for analysis.

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X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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PI Signature 2-14-00 Date

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Introduction

It is well established that covalent binding of chemical carcinogens to DNA is the initiating event in carcinogenesis. While exposure to environmental contaminants in the air, food, and especially from cigarette smoke may result in significant levels of DNA damage, it has not been proven that these exposures can influence the development or initiation of breast cancer. This project investigates the role of polycyclic aromatic hydrocarbons (PAH)-DNA adducts in the etiology of breast cancer by taking advantage of an ongoing study, the Long Island Breast Cancer Study Project (LIBCSP). The parent LIBCSP is determining the level of PAH-DNA adducts in lymphocytes of cases and controls by Enzyme Linked Immunosorbant Assay (ELISA). The studies ongoing in this project use an immunofluoresence method to measure PAH-DNA in breast tissue of cases and in lymphocytes of cases and controls. An aim of the study is to correlate lymphocyte DNA damage with breast tissue damage, in order to validate the use of blood as a surrogate marker for tissue in future studies. A correlation will be made between the levels of adducts in lymphocytes detected by two different assays ELISA and immunohistochemical detection. A good correlation would justify the use of the immunohistochemical method and hence the necessity for less blood from subjects in future studies. This study will test the hypothesis that women with dietary and lifestyle (e.g. smoking) exposure to PAHs will have higher levels of PAH-DNA adducts in breast tissue and lymphocytes and that the subjects with the highest degree of adducts have a variant allele in their DNA repair gene XPD. The demonstration that this biomarker

(PAH-DNA) is indicative of exposure and related to breast cancer will contribute to chemoprevention. Showing that a specific allele is asociated with increased DNA damage, presumably due to a decrease in a women's ability to repair these adducts, would help in our understanding of what groups are at a higher risk than others for breast cancer.

BODY

9/97-8/98

The majority of my first year was spent preparing the slides that will be used to compare breast cancer cases lymphocytes with controls from the parent study, the Long Island Breast Cancer Study Project(LIBCSP). As cells were thawed for DNA isolation, I took 10 *ul* of lymphocytes and smeared cells on a precoated slide. I have four slides prepared for each subject. I will only use one, but the other three were prepared for future studies on this population. The slides were then fixed using ethanol and stored with a database that has date and location of the slide and the subject's number for my study and for future use. This involved the preparation and entering of >4000 slides.

While I was preparing slides, I was also establishing the methodology that will be used to stain the 800 final slides. Experimentation was done to decide between the use of a monoclonal or polyclonal antibody and the use of immunoperoxidase versus immunoflourescence method. (See Table 1) I grew MCF-7 cells, then treated them for 2 hours with benzo(a)pyrene diol epoxide (BPDE) in order to induce adducts in the cells. MCF-7 cells were grown in dishes. Half the plates' growth medium was treated with BPDE, which induces high levels of damage as this metabolite of benzo(a)pyrene is the most carcinogenic. The other half of the plates were given the normal growth medium plus THF (Tetrahydrofuran) the solvent used for BPDE. After trypsinization, cells were smeared onto slides and fixed. By immunohistochemical analysis, it was clear that the cells treated with BPDE had many more adducts than the ones treated with only solvent. With the use of MCF7 cells either treated or not with BPDE as controls, I obtained up to a 20 fold difference using the immunofluorescence method with the polyclonal antibody.

This was the best result I obtained, so this antibody, Polyclonal #29, with a secondary antibody conjugated to FITC will be used for the entire study. Tests were done on some of the lymphocyte samples to determine the best dilutions of my primary and secondary antibody to get low background staining and the best reproducibility of the data.

9/98-11/99 A study to determine the effects of a polymorphism in XPD, a DNA repair gene, in breast cancer.

While awaiting delivery of a new CCD camera and software for quantitation of immunostaining, I was unable to immediately start the staining and quantitation of fluorescence of the slides. Therefore, I began a project not written in the original proposal of the grant. A DNA repair project was begun investigating a new polymorphism found in XPD (Jones, 1998). It results in a non-conservative amino acid change from A to C at position 36931 in exon 23 resulting in a change from Lys-Gln. The functional significance of this change has not yet been shown, however there is high sequence conservation among species for this gene. A 98% homology of this gene from hamster to human is indicative of a functional role for this region of the gene. XPD belongs to the nucleotide excision repair pathway and is required for the removal of bulky adducts, like DNA adducts, by excising a 24-32 nucleotide single-strand oligomer containing the adduct. It seems biologically plausible that a change in structure or function of this gene could cause a change in the effectiveness of repair of the adducts that I am measuring from the subjects of the LIBCSP. Using the DNA of all 800 of the same subjects I will assay for PAH-DNA adducts, I amplified a piece of this gene from

exon 23, digested it using *PST*1 to determine the allele frequency and performed gel analyses of 1055 samples. This included a 10% quality control duplication after blinding. This study complements the originally funded proposal and will allow us to investigate a gene-environment interation that results in carcinogenesis. I have finished the laboratory work for the DNA repair project. However, my thesis committee has indicated that I should not analyze the results to remain blinded to the status of cases versus controls. To un-blind me for the DNA repair gene study before I finish the analysis of the immunofluorescence could create bias. Therefore, it is important that I not do the analysis myself on the DNA repair data to keep the study as unbiased as possible. After I finish generating my immunofluorescence data, I will be un-blinded to the status of cases and controls and analyze all of the data. These analyses will include the primary hypothesis of this study outlined in the introduction and also other questions involving the interindividual variation in genes and their possible contribution to cancer risk. By using adducts as my marker for exposure versus using questionnaire data I have already taken into account variability in the metabolism of the PAH carcinogens. I will now also have data on the DNA repair capability of the subjects which will help us to understand why cancer risk is so variable even among supposedly homogenous populations. In adddition to the original data analysis which included four aims;

I. Test the hypothesis that women with dietary and lifestyle exposure to PAH will have higher levels of adducts in the breast tissue and in lymphocytes than in those with lower exposure.

II. Establish lymphocytes as a surrogate for target breast tissue.

III. To correlate the levels of adducts found by immunofluorescent detection with the ELISA method.

IV. To assess the association between breast cancer and PAH-DNA adducts.

The following analyses will be added;

I. Does a specific allele type (polymorphism) in exon 23 of XPD have a higher frequency in cases compared to controls?

II. Does the polymorphism affect DNA adducts?

Reportable outcomes

Table 1

Comparison between the immunoperoxidase and immunofluorescence methods and polyclonal to monoclonal antibodies for the detection of BPDE-DNA addducts.

	Relative Staining Intensity		
Monoclonal antibody 5-D-11	BPDE	Control	Ratio
Immunoperoxidase	37.8	15.4	2.45
Immunofluorescence	105.1	57.5	1.83

Polyclonal Antibody 29	BPDE	Control	Ratio
Immunoperoxidase	100	34	2.94
Immunofluorescence	1249	58	21

Conclusions and Perspectives

While the completion of the quantitation of fluorescence of the 800 slides has been delayed, this study has, in its first year, been productive due to the addition of some exciting new aims for the study. The original Statement of Work has been changed from my grant proposal but my new time line will reflect more work and still stay on schedule for the completion of this grant. By the end of this year, it is projected that all the immunofluorescent staining will be completed (800 lymphocyte samples, 150 breast tissue samples). The analysis of the data and writing any papers that come from this data will be completed in the last year of the grant.

References

1. Shen RM, Jones IM, Mohrenweiser H. (1998) *Cancer Research* 58 , 604-608.
2. Santella RM, Lin CD, Cleveland WL, Weinstein IB. (1984) *Carcinogenesis* 5, 373-377.
3. Poirier MC, Santella RM, Weinstein IB, Grunberger D, Yuspa, SH (1980) 40, 412-416.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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MCMR-RMI-S (70-1y) 28 Feb 03

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FOR THE COMMANDER:

PHYLLIS M. RINEHART
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