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Molecular Biology and Prevention of Endometrial Cancer

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14. ABSTRACT
Objective: To increase our understanding of the molecular aberrations associated with endometrial carcinogenesis and the biologic mechanisms underlying the protective effect of oral contraceptive (OC) therapy. Methods: 1) Oligonucleotide microarray analysis was performed on a panel of endometrial cancers. 2) A subset of adenocarcinoma cases from the International DES Registry (IDESR) was analyzed for MSI 3) A case-control study of the CASH database was performed to evaluate the relationship between progestin potency and endometrial cancer risk. 4) An analysis of endometrium samples from cymologous macaques that were exposed to long term progestins was performed. 5) A clinical trial comparing progestin versus placebo is underway that will facilitate investigation of the effects of progestin exposure on the endometrial lining. Results: 1) Different histological types of endometrial cancer have unique genomic expression patterns. 2) The poor quality DNA from the majority of IDESR samples prohibited an adequate analysis of the case set. 3) A case-control study has suggested higher progestin-potency OCs may be more protective than lower progestin potency OCs among women with a larger body habitus. 4) Macaque studies have suggested that induction of apoptosis may be a mechanism underlying the chemoprotective effects of progestin on the endometrium. 5) Regulatory hurdles have resulted in delays in initiation of the clinical trial which is now currently underway.

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Endometrial cancer, hormone, prevention

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INTRODUCTION

Endometrial cancer is the most common type of gynecologic cancer in the United States and was estimated by the American Cancer Society to have been newly diagnosed among 40,000 American women in the year 2004 and lead to approximately 6500 cancer related deaths (1). Approximately 25% of all endometrial cancers occur in premenopausal women (2). Major advances in our understanding and treatment of endometrial cancer have occurred over the past decade, yet the frequency of this cancer in the general population has not been altered appreciably. Despite the known protective effect of oral contraceptives, little has been learned regarding the underlying mechanism. We believe that an understanding of the molecular profiles of endometrial cancers and the molecular events underlying the protective effect of oral contraceptives against endometrial cancer could facilitate the development of effective chemopreventives and significantly decrease the incidence of endometrial cancer in women.

BODY

Aim 1: To characterize and compare the molecular profiles of Type I endometrioid endometrial cancers, which often develop in an estrogen milieu, to that of Type II endometrial cancers. In addition, we will use microarray to examine the molecular changes in the endometrium associated with progestin exposure in order to gain insight into the biologic mechanism underlying the chemopreventive effect of the oral contraceptive pill (OCP).

Project 1: Objectives completed and data previously submitted with 2004 report. Data published this past year and listed in “Reportable Outcomes”.

Project 2 (Pending): We requested a third no cost extension (NCE) on 5/21/07 for proposal No. 0155012, Award No. DAMD17-02-1-0183 that will involve Walter Reed Army Medical Center, Wake Forest University, and Evanston Northwestern. Our group has had significant delays in approval of the human trial outlined in Project 4, Study 1 (“The Chemoprotective Effects of Progestin on the Endometrial Lining”). We final received approval for HSRRB #A-11191.2b on 3/21/05. Unfortunately, during the interim of protocol review and approval locally and regionally (between 11/29/01 and 3/21/05), the manufacturer (Wyeth) of the study drug (Ovrette) had discontinued sales of the drug in the United States. After consideration of multiple options disclosed in last years NCE, we ultimately located a supplier of the drug and Analytical Research Laboratory (Oklahoma City, OK) and Northpointe Pharmacy (Oklahoma City, OK) were chosen as the testing laboratory and formulating pharmacy. Method development of the various analytical tests was commenced in January 2007, release testing of the finished capsules began in May 2007, and stability testing began in June 2007. Results from the analytical tests and formulation process were forwarded with our IND application which was approved by the FDA 8/28/07. Once the IND was approved, the amended documents outlining the changes made to
the original approved protocol by using a formulated drug was resubmitted to the WRAMC DCI on 9/27/07. The amendment was approved with minor changes on 12/13/07 and a stamped consent and HIPPA form was provided by DCI on 12/19/07. All documents were subsequently forwarded to Ft. Detrick and an approval letter from Ft. Detrick was issued on March 3, 2008. At this time, WRAMC has commenced the drug/placebo and patient enrollment efforts; however, all amended protocol documents are awaiting review/approval by the Institutional Review Board at Evanston Northwestern Healthcare (ENH). After speaking with the site P.I. at ENH, Dr Rodriguez, this review and expected approval should occur within the next month.

We recently received a no-cost extension of twelve months to continue our research efforts at Walter Reed and Evanston Northwestern. We propose utilization of our existing funds to provide consumables used in the enrollment of patients as well as collection of tissue and data involved in the completion of the randomized controlled trial. Other nursing personnel resources associated with support of this project will be provided by the Division of Gynecologic Oncology at both ENH and Walter Reed.

The IRB approved protocols for the randomized clinical trial in DAMD17-02-1-0183 provided permission for both primary analyses of the tissues as well as banking of specimens for future second use analysis. Our group already has approval for secondary use of the specimens (to be collected) with analysis of tubal and ovarian tissue in the program project entitled “Gynecologic Disease Program” (W81XWH-05-2-0005). Our proposed plan would be to perform both the primary and secondary analysis of tissues collected as part of the current trial within the “Gynecologic Disease Program”.

Over the past year, our program has delays in recruitment related to change over in research nursing staff personnel that prompted us to delay initiation of recruitment at both sites. Now that these changes have been implemented, we are currently underway with enrollment and have requested a no cost extension to allow us time to complete the study.

Aim 2: To analyze vaginal and cervical adenocarcinomas, that have arisen in women exposed to DES in-utero, for methylation and mutation of PTEN and MLH1 in order to determine if estrogen induces genetic alterations in these tumors characteristic of Type I endometrioid carcinomas. Although a pilot study aimed at an analysis of MSI in 7 cases from the International DES Registry was successful with repetitive attempts at DNA amplification, the analysis of the entire set was not successful presumably secondary to the quality of the DNA which reflects the old age of the specimens and the various methods that were used in their preservation. Less than 50% of the samples amplified at any one of the markers making the data inadequate for designation of MSI status. Significant amounts of material from the Transplacental Registry were used unsuccessfully to complete the work on the microsatellite instability. Acquisition of additional material to further evaluate alterations in either mismatch repair genes (causative of MSI) or PTEN was not an option. The inability to pursue this aim further was previously described in the 2006 annual report.

Aim 3: Using data from the Centers for Disease Control Cancer and Steroid Hormone Study, we will determine if the protective effect of OCP’s against endometrial cancer are impacted by the progestin or estrogen potency of OCP formulations. Objectives completed and data were previously submitted with 2004 report. Published manuscript were listed in the 2004 “Reportable Outcomes”
Aim 4: To test the hypothesis that the oral contraceptives and hormone replacement therapy progestins provide a chemoprotective effect against endometrial cancer through induction of apoptosis, PTEN, and TGF-beta in the endometrium.

Epidemiological studies have demonstrated that OCP use lowers the risk of subsequent endometrial and ovarian cancer. Although the biologic mechanism(s) underlying the protective effect of OCP’s on the risk of both of these cancers have not been well defined, there is evidence to suggest that biologic effects related to the progestin component may underlie the cancer preventive effects of the OCP. Recent studies have reported the progestin-mediated activation of apoptosis in endometrial cancer cell lines and endometrial hyperplasias. The finding that progestin activates the apoptosis pathway in endometrial cells raises the possibility that this may be a major mechanism underlying the therapeutic effect of progestins against endometrial hyperplasia. Similarly, our group has found that progestins markedly activate both apoptosis and TGF-beta expression in the ovarian epithelium leading to the hypothesis that progestins may act as chemopreventives for ovarian cancer. It is interesting that tumors arising from the ovary and endometrium share common epidemiological risk factors, and that both the endometrium and ovarian surface epithelium share a common embryological precursor. It is thus plausible that progestins activate similar molecular pathways relevant to cancer prevention in both of these organ sites. Recent evidence suggests that expression PTEN appears to be upregulated in the secretory phase of the menstrual cycle. It is plausible that the chemopreventive effects of OCP’s are mediated through overexpressed PTEN with resultant suppression of cell cycle progression and activation of apoptosis in endometrial cells.

- The short-term effects of progestins on apoptosis as well as the expression of PTEN and TGF-β in the endometrium will be evaluated using uterine specimens collected from patients enrolled in a double-blinded prospective randomized trial. See Aim1, project 2 for explanation of delays in deliverables and current “no cost extension status”.
- The long term effects of progestins on apoptosis as well as the expression of PTEN and TGF-b in the endometrium were evaluated using uterine specimens from cynomolgus macaques (80 premenopausal and 130 postmenopausal) previously part of a three-year randomized trial designed to evaluate the effects of the combination oral contraceptive pill and hormone replacement therapy on reproductive organs. This objective has been completed and the results were submitted with our June 2007 annual report.

KEY RESEARCH ACCOMPLISHMENTS

- **Aim 1:** Identified genes that are differentially expressed between endometrioid and papillary serous endometrial carcinoma and determined that histology can be predicted on the basis of gene expression in approximately 90% of cases. Identified additional genes that are differentially expressed in endometrial cancer vs. normal endometria. Confirmed that microsatellite stable endometrial cancers have unique gene expression profiles compared to those with microsatellite instability.
- **Aim 3:** Established that progestin containing oral contraceptives (OCs) are associated with a decreased endometrial cancer risk and that higher progestin- potency OCs may be more protective than lower progestin potency OCs among women with a larger body habitus.
• Aim 4: Completed analysis of apoptosis and TGF using endometrium specimens from macaques exposed to various hormonal regimens and found evidence to explain the chemoprotective effects of progestin on the post-menopausal endometrial lining

REPORTABLE OUTCOMES (since last report)

• None

CONCLUSIONS

Different histological types of cancer have genomic expression patterns that reflect unique pathways of carcinogenesis. Likewise, cancers characterized by microsatellite instability result in the expression of genes most likely to be affected by alterations in mismatch repair. As we improve our understanding of the alterations that accompany endometrial carcinogenesis, it is likely that future chemopreventives may be developed for several types of endometrial cancer, each of which develops by specific pathways. In regards to contemporary chemopreventive options, an analysis of data from the CDC CASH database has suggested that a greater protective effect against endometrial cancer may be associated with high progestin potency OCs, particularly in patients with a larger body habitus. Higher potency progestin containing OCs should be considered in forthcoming endometrial cancer prevention trials particularly if other studies suggest a greater risk reduction associated with heavier women that are at highest risk for endometrial cancer. Using a macaque model, we have determined that the mechanisms behind the chemoprotective effects of progestin containing hormonal regimens in post-menopausal patients appear to be in part related to induction of apoptosis. We look forward to evaluation of the short term effects of progestin containing hormonal formulations in our clinical trial evaluating the short term effects of progestin on the endometrium lining using both a targeted analysis of apoptosis as well as an assessment of global gene expression using oligonucleotide microarray.

REFERENCES