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Molecular Detection of Circulating Cancer Cells for Early Diagnosis of Breast Cancer

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Goal: The goal of this project is to detect circulating cancer cells using breast-specific tumor markers in the blood from 200 patients who have suspicious mammograms and breast tissue biopsies. These molecular detection results will be correlated with the biopsy results.

Patient accrual: Because this study just recently opened in July, 2001, we have collected blood from 54 patients with suspicious mammograms immediately prior to a breast tissue biopsy. These patients were informed of the investigational aspects of this study and have given written consent in accordance with institutional and federal guidelines.

Molecular detection progress: Isolation of mononuclear cells by density gradient centrifugation and immunomagnetic capture of epithelial cells from all the collected blood samples have been performed. Isolation and reverse transcription of mRNA from each accrued sample are currently in progress. After reverse transcription, cDNA from each sample will be amplified using fluorescent-based kinetic PCR with gene-specific primers.

Circulating cancer cells will be detected using mamoglobin, B305D, and B726P, which are breast-specific genes, and γ-aminobutyrate type A receptor β subunit, cytokeratin-19, and β-actin.

Summary: We have collected 25% of the proposed 200 sample accrual, and the molecular detection of circulating cancer cells from the collected samples is in progress.
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INTRODUCTION

Circulating cancer cells are present in the blood of breast cancer patients at a very early stage (1, 2). Early detection is the most significant means for reducing the morbidity and mortality due to breast cancer. In addition to mammography and physical examination, sensitive molecular techniques may also be used to detect early stage breast cancer. Detecting circulating cancer cells in the blood using a panel of genes that display complementary expression can be used as a sensitive screening tool for breast cancer. If validated, this test would detect patients with breast cancer while decreasing the number of unnecessary biopsies and false negative mammographic results. We hypothesize that sensitive molecular detection of cancer cells in peripheral blood using novel breast-specific genes will provide a screening test that can be used independently or in concert with mammography and physical examination to more accurately detect early stage breast cancer. The goal of this project is to detect circulating cancer cells in the blood from a population of patients who have suspicious mammograms (200 patients) using breast-specific tumor markers. These results will be correlated with the breast biopsy results. We have developed an immunomagnetic cell capture technique that is coupled with RT-PCR for detection of breast cancer cells in blood and bone marrow (3). Unique, breast-specific genes with complementary expression in 100% of breast cancers will be used (4). This study will determine the clinical significance of circulating cancer cells in a select population of patients. Once sensitivity and specificity is established, a screening test will be validated in future studies with an appropriate population of patients.

BODY

In this study, molecular detection techniques are used to detect circulating cancer cells in the blood of patients with suspicious mammograms who are undergoing breast biopsy. Our method is extremely sensitive and in a model test system using MDA-MB361 breast cancer cells, it is capable of detecting one breast cancer cell in 10^7 nucleated cells.

Research Accomplishments

Patient accrual:

This study opened in July 2001, and we have collected to date blood samples (20 ml) from 54 patients (proposed accrual number is 200). Six of these samples were study cancels because either the patients had a prior history of cancer or fine needle aspirations were performed. All patients were informed of the investigational aspects of this study and provided written consent in accordance with institutional and federal guidelines. All blood samples were assigned a unique identification number.

Molecular detection methods and progress:
1) Density gradient centrifugation to isolate mononuclear cells from the blood.
2) Immunomagnetic capture of epithelial cells with the monoclonal antibody, BER-EP4, using the magnetic Dynabeads Epithelial Enrich kit (Dynal A.S., Oslo, Norway).
   Within one day of each blood draw, we isolated mononuclear cells by density gradient centrifugation and captured epithelial cells from all the collected blood samples.
3) Isolation of mRNA using the mRNA Direct kit and DynaBeads Oligo (dT)25.
4) Reverse transcription of mRNA with random hexamers.

The isolation and reverse transcription of mRNA from each accrued sample are currently in progress. Reverse transcription of the mRNA attached to the Dynabeads Oligo (dT)$_{25}$ results in a first strand cDNA that is covalently attached to the beads (solid-phase cDNA library). The solid-phase cDNA library can be used repeatedly with specific 5’or random hexamer primers and Taq polymerase to synthesize second strand cDNA, which serves as a template for quantitative real-time PCR (5, 6).

5) PCR with gene-specific primers using real time PCR in an ABI 7700 prism Taqman instrument (PE Biosystems, Foster City, CA).

The second strand cDNA from each sample (stored at 4°C) will be amplified using fluorescent-based kinetic PCR with gene-specific primers. Circulating cancer cells will be detected using mammaglobin, B305D, and B726P, which are breast-specific genes, and γ-amino butyrate type A receptor π subunit (GABA A$_{\pi}$), cytokeratin-19, and β-actin. The PCR assays using Taqman™ chemistries (7) for each gene have been successfully developed in our laboratory. Matching primers and fluorescent probes have been designed (Primer Express Program, Applied Biosystems, Foster City, CA) and obtained for each of the genes. Plasmids containing the gene of interest were constructed using the original TA Cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. These plasmids will be used in the real-time PCR assays to generate standard curves, which will allow for quantification of gene expression. The presence of β-actin will be used as an endogenous RNA control in the samples to normalize the gene expression of mammaglobin, B305D, B726P, and GABA A$_{\pi}$.

In summary, we have collected approximately 25% of the proposed 200-sample accrual, and the molecular detection of circulating cancer cells from these samples is in progress and approximately 30% complete. This study will determine the clinical significance of detecting circulating breast cancer cells in a select population of women and will establish its potential utility as a screening test for breast cancer.

**KEY RESEARCH ACCOMPLISHMENTS**

- Processed all blood samples collected to date.
  - Isolated mononuclear cells from the blood.
  - Immunomagnetic captured epithelial cells.
- Successful development of PCR assays using Taqman™ chemistries for each gene.
- Successful construction of plasmids containing the genes of interest.

**REPORTABLE OUTCOMES**

This study is only partially completed in which no manuscripts or abstracts have been reported. It is anticipated that we will be able to report some interim results in the spring of 2002.

**CONCLUSIONS**

In summary, we propose that this study will determine the clinical significance of detecting circulating breast cancer cells in a select population of women and will establish its potential
utility as a screening test for breast cancer. This study is approximately 20% complete with anticipated interim results being reported in the spring of 2002.

Because we have collected approximately 25% of the proposed 200-sample accrual, we are in the process of including the Mayo Clinic in Jacksonville, FL to participate in this study with Dr. Edith Perez as co-principal investigator in Jacksonville. This will aid in accruing additional samples for this study in anticipation that the accrual number of 200 will be obtained before the study completion date of October 2002. I have been in contact with Ms. Maryann Pranulis about Jacksonville’s participation and I am currently pursuing the collection of the pertinent information needed for the DOD (e.g., Dr. Perez’s CV and Jacksonville’s consent form and IRB approval notification).

REFERENCES


APPENDICES

None