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TITLE: Biomarker Analysis on Ductal Lavage Fluid as a Tool for
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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*
The current breast cancer diagnostic methods rely on identification of the tumor mass and therefore have limited success for detecting early lesions in which preventive measures could effectively be carried out. We propose a novel approach using ductal lavage technique, coupled with biomarker profile analysis on the fluid and cells obtained. This approach facilitates the detection of malignant or premalignant lesions in individuals who have undetectable tumor masses. We hypothesis that the development of breast cancer is preceded by molecular field disease changes, and therefore cells obtained from any area of the duct will signal the biochemical and molecular changes associated with carcinogenesis. To test this hypothesis, we first performed a molecular mapping study. Ductal epithelial cells obtained from cancereous and non-cancerous ducts were collected from several mastectomy samples. Molecular analysis including analysis of proliferation marker Ki67 and methylation of RAR-B were performed. The results showed that lavaged ducts as well as adjacent non-canerous duct epithelial cells shared similar molecular abnormalities to the cancerous duct epithelial cells. This finding provided direct support for the field disease concept of breast cancer carcinogenesis and further substantiate the ductal lavage technique as an approach for breast cancer early detection. In our pilot study, ductal lavage was performed on contralateral non-cancer breast for 3 patient with newly diagnosed breast cancer, 10 patients with atypical breast lesions with nipple discharge, 9 patients with benign breast lesions without nipple discharge, and 19 healthy female with family history of breast cancer in both Chinese women and American women. The results showed that ductal lavage was capable of detecting abnormal cells from high risk individuals without clinically identifiable lesions, thus provided a means for early detection. Further studies are needed to confirm the observation.

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breast cancer, ductal lavage fluid, Ki67

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Table of Contents

INTRODUCTION

The current breast cancer diagnostic methods rely on identification of the tumor mass, and therefore have limited value for detecting early lesions in which preventive measures could effectively be carried out. Unlike other solid organs, the human female breast consists of mostly fatty tissue, and random sampling using fine needle aspiration or core biopsy for a unpalpable, even a radiological or clinically suspected lesion, is usually unrevealing.[1] Techniques that can directly obtain cells from the duct epithelial cells (which are the cells of most of breast cancers originated from) and methods that can analyze multiple biomarkers on small volume basis are therefore urgently needed. Recently two emerging techniques, the ductal canalization and Biomarker Profile Analysis, have been developed exactly for such purposes. This study is designed to test the feasibility of combining these two techniques together and determine preliminary whether such an approach provides a more accurate screening test to detect earlier breast lesions than the existing mammogram technique.

The duct lavage technique developed by ProDuct Health, Inc. allows the sampling of cells from a specific area of the breast duct, and is highly efficient in obtaining cells for analysis.[2] The Biomarker Profile Analysis is an approach developed in our laboratory. This method enables the analysis of multiple phenotypic and genotypic biomarkers on a small sample volume basis, without the need to compromise the conventional cytomorphologic analysis.[3,4] However, since each breast contains 7 to 12 ducts, and it is unrealistic to canalize all the ducts in clinical setting, it is crucial to determine whether specific molecular changes associated with breast cancer can be detected in the "field". And if so, then biomarker profile analysis on cells obtained from one the field might provide information to determine whether there is an early malignancy elsewhere. Therefore an additional purpose of this pilot study is to test such "field" hypothesis.

This study was carried out in small groups of women who have various risks of developing breast cancer. The studies will be performed in Chinese women in Beijing, China and American women in Los Angeles. The recent rapid increase of breast cancer incidence in Chinese women provides a unique opportunity to study the biology of breast cancer development. The rational to involve Chinese women in China is mainly because the ductal lavage device has not been tested in Asian women, including Chinese women, and in addition we would like to test the feasibility of conducting such study in China. The ultimate goal of this project is to develop a simple, minimally invasive, and accurate screening method for breast cancer. The immediate objective of this project is to determine the feasibility of performing ductal lavage analysis in women with different racial and social environmental backgrounds, and study preliminary the values of such an approach in breast cancer early detection.
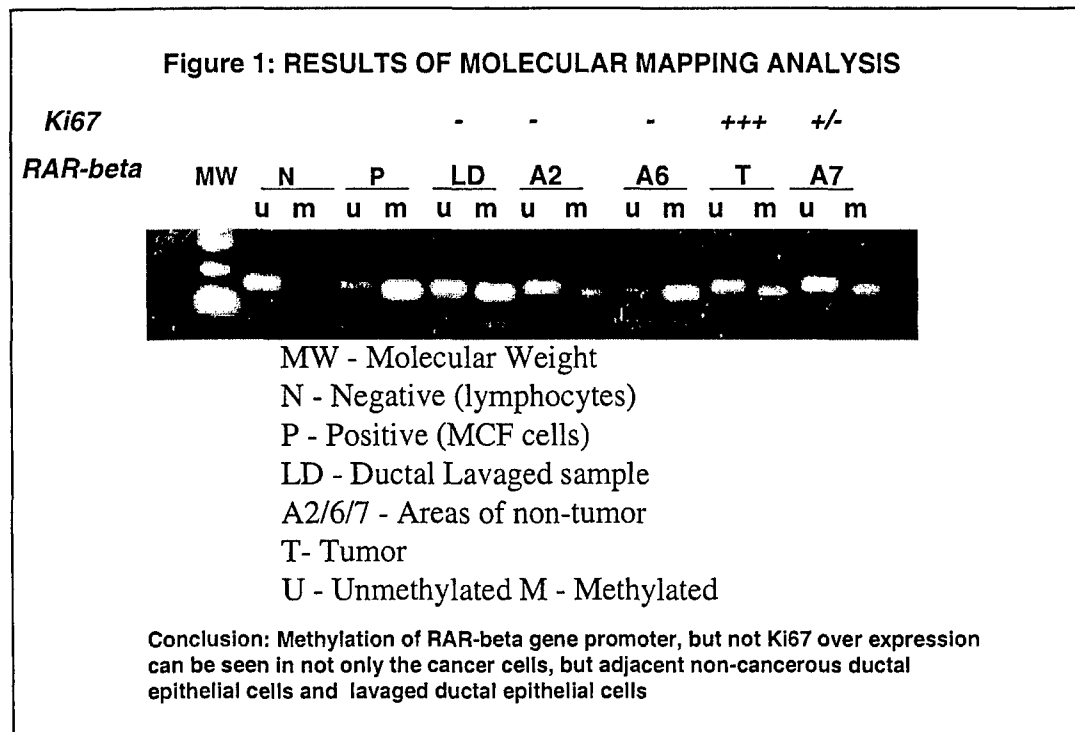
BODY

Specific Aims:

There were two specific aims in this pilot study:
1. To perform a molecular mapping analysis to further validate ductal lavage approach and to identify novel molecular markers
2. To perform lavage in both Chinese and American women, to test the feasibility of performing ductal lavage in women with different ethnic and social background

Results:

For specific aim #1, mastectomy specimens were used to obtain ductal epithelial cells by microdissection from lavaged ducts, adjacent non-cancerous ducts, and cancerous ducts. Markers analyzed included Ki67 by immunohistochemistry and RAR-beta by methylaiton specific PCR. RAR-beta is a marker for cellular differentiation and a tumor suppresser gene while loss of function seen in breast cancer, primarily by mechanisms of DNA hypermethylation and LOH. Methylation of RAR-beta-2 can be detected in ductal fluid obtained by ductal lavage technique.[5] As shown in Figure 1 below, our study demonstrated taht methylation of RAR-beta gene promoter, but not Ki67 over expression can be seen in not only the cancer cells, but also adjacent non-cancerous ductal epithelial cells and lavaged ductal epithelial cells. This finding supports our hypothesis that cancer associated molecular "field defect" can be detected in non-cancerous ductal epithelial cells, thus may be potential biomarkers for breast cancer risk.

Figure 1: RESULTS OF MOLECULAR MAPPING ANALYSIS

MW - Molecular Weight
N - Negative (lymphocytes)
P - Positive (MCF cells)
LD - Ductal Lavaged sample
A2/6/7 - Areas of non-tumor
T- Tumor
U - Unmethylated M - Methylated

Conclusion: Methylation of RAR-beta gene promoter, but not Ki67 over expression can be seen in not only the cancer cells, but adjacent non-cancerous ductal epithelial cells and lavaged ductal epithelial cells

For Specific Aim #2, a pilot study was performed in both Chinese and American Women. The study groups were:
   In Cancer Institute/CAMS, China:
1. Contralateral non-cancer breast for 3 patient with newly diagnosed breast cancer
2. 10 patients with atypical breast lesions with nipple discharge
3. 9 patients with atypical breast lesions without nipple discharge

   In UCLA:
4. 19 healthy female with family history of breast cancer.

Cytological analysis was performed on Thin prep slides, and the results were presented in following table:

Overall our preliminary study showed that cytological analysis of ductal lavage samples correlated well with breast cancer risk. Severe cytological atypia was detected in the contralateral breast in one of three breast cancer patients, and 1 of 10 patients with atypical breast lesions (with atypical ductal hyperplasia to atypical lobular hyperplasia), but none in women with benign breast lesions (fibroadenoma) and healthy individuals but with positive family history.

Study #2: Ductal lavage analysis in subjects with various risk of developing breast cancer - Results of a pilot study

Subject Group	n	Attempted Lavaged Ducts	Succesfully Lavaged Ducts	Cytological Findings
1. Contralateral breast in newly dig. breast ca patient	3	5	4	1 - Severe atypia 2 - Mild atypia 1 - Negative
2. Atypical breast lesion with nipple discharge*	10	10	10	1- Severe atypia 6 - Mild atypia 3 - Negative
3. Benign breast lesions without nipple discharge	9	9	5	1- Mild atypia 4- Negative
4. Healthy individual with family history	19	24	13	1-Mild atypia 12- Negative

KEY RESEARCH ACCOMPLISHMENTS

1. IRB approval from all levels
2. Both video and in person training for performing ductal lavage in Cancer Institute/CAMS, China
3. Experience gained from mastectomy specimen before live patient
4. Provided further molecular evidence to support ductal lavage approach
5. Demonstrated the feasibility of ductal lavage procedures in women with different ethnic and social environmental backgrounds
6. Molecular based analysis, including QFIA analysis as proposed, of collected material is still ongoing

REPORTABLE OUTCOMES

1. Cytological analysis of ductal lavage fluid from women with various risk of developing breast cancer

CONCLUSIONS
1. Ductal lavage provides an useful alternative approach for early detection of breast cancer
2. Feasibility to perform international collaborative ductal lavage study is demonstrated
3. Additional studies will be needed to determine the value of biomarker analysis in lavage samples

REFERENCES

[1] Salami, N, Hirschowitz, S, Nieberg, R, Apple, S. Triple test approach to inadequate fine needle aspiration biopsies of palpable breast lesions. Acta Cytologica, 43(3):339-43, 1999.

[2] Love, SM; Barsky, SH. Breast-duct endoscopy to study stages of cancerous breast disease. Lancet, 348(9033):997-9, 1996.

[3] Rao, JY, Apple, S, Jin, YS, Hemstreet, GP, and Nieberg, R. Single cell multiple biomarker analysis in archival Fine Needle Aspiration specimens: Quantitative Fluorescence Image Analysis of DNA ploidy, p53 and G-actin as breast cancer biomarkers. Cancer Epidemiology, Biomarkers and Prevention, 7(11)1027-1033, 1998.

[4] Rao, JY, Apple, S, Jin YS, et al. Comparative PCR analysis of c-myc amplification on archival breast fine needle aspiration (FNA) materials. Cancer Epidemiology, Biomarkers and Prevention. 9:175-179, 2000.

[5] Evron E, Dooley WC, Umbricht CB, Rosenthal D, Sacchi N, Gabrielson E, Soito AB, Hung DT, Ljung B, Davidson NE, Sukumar S. Detection of breast cancer cells in ductal lavage fluid by methylation-specific PCR. Lancet. 28;357(9265):1335-6, 2001.