BENIGN BREAST TISSUE AS A “CRYSTAL BALL”:
USING MUTATIONS TO PREDICT BREAST CANCER RISK
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Abstract Form

Introduction: Annual age-adjusted breast cancer incidence rates in the United States have been static for decades and are a testament to the lack of effective prevention strategies. Few, if any, of the current interventions are based on an understanding of how breast cancer risk is transduced at the molecular level.

Cancer is thought to occur as a consequence of the progressive accumulation of several somatic mutations. We hypothesized that the mutations present in the benign breast biopsies of women, who went on to develop breast cancer years later, are different from those who did not develop this disease and these mutations could be used as markers of the risk of development of breast cancer.

Methods: A case-control design was chosen to test the hypothesis. As a preliminary feasibility study, a total of 24 cases and 11 controls were selected for DNA sequencing. Exome libraries were sequenced (100 base pairs, paired-end) on an Illumina HiSEQ2500.

Results and Conclusions: 50370 nucleotide variants were identified that occur either in splicing sites or exons. Following selection for rare variants, a two-proportional z-test was performed and a p-value was obtained. 705 variants with p-value < 0.10 were retained. The variant list was annotated using algorithms that predict the effect of the variant on protein structure and function. Among these 705 variants, 397 variants across 369 genes were nonsense, splicing, or deleterious nonsynonymous variants. The variants were cross-referenced with lists of known driver mutations and with the Sanger COSMIC cancer Gene Census, which catalogues those gene mutations causally implicated in cancer. Non-matrix factorization (NMF) was performed to reduce the dimensionality of the data. Following NMF decomposition, coefficient and basis matrices were retained to train a classifier to distinguish cases and controls.

The classifier was able to distinguish the cases and controls accurately. In a cross-validation test using three algorithms for scoring the deleteriousness of single nucleotide variants, CADD
(Combined Annotation Dependent Depletion), SIFT (Sorting Intolerant From Tolerant), and PP2 (PolyPhen-2), as annotation tools, the average prediction accuracy was $86.91\% \pm 1.6\%$, $85.29\% \pm 1.9\%$, and $77.94\% \pm 2.5\%$; the average AUC score was $0.95 \pm 0.02$, $0.94 \pm 0.02$, and $0.85 \pm 0.02$, respectively. This study presents a generic and complete process to study the associations between whole-exome sequencing data and breast cancer occurrence. The newly discovered genetic variants associated with cases can lead to biological insights regarding oncogenesis.
CHARACTERIZATION OF BREAST DUCTS AND DUCTAL EPITHELIUM
IN WOMEN AT RISK FOR BREAST CANCER

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Breast cancer develops through the accumulation of genomic changes in breast ductal epithelium. An understanding of these changes and their association with risk for breast cancer is important for developing new molecular profiles of risk assessment, identification of new targets for breast cancer prevention, and understanding the carcinogenic pathway for breast cancer. Recent studies have indicated the presence of genomic changes in normal breast tissue in women at normal risk for breast cancer, including loss of heterozygosity, DNA methylation, and telomere shortening, and progression of these changes in normal breast tissue in women at high risk for breast cancer. These changes are associated with the development of large cancerized fields with decreased genomic stability in the breast. Together these findings suggest a pattern for development of genomic changes in the preneoplastic carcinogenic pathway of the breast. We have complimented these findings with an ongoing clinical trial evaluating cytologic ductal cellular changes, structural changes with ductal endoscopy, and molecular profiling of ductal epithelium in women at normal risk and at high risk for sporadic breast cancer. This study resulted in the development of a new ductal sampling technique which significantly improved epithelial cell yield, cell homogeneity, and molecular profiling of both normal risk and high risk women. Multiple ductal fluid and ductal cell samples, with corresponding demographic and risk assessment information, is available for each subject. We found cytologic epithelial atypia was present in both normal risk and high risk subjects and was accompanied by benign changes on ductal endoscopy. The improved ductal sampling technique provides cellular and ductal fluid samples suitable for a wide range of RNA and DNA molecular profiling studies as well as the development of human mammary epithelial cell lines. Together these findings indicate the feasibility of clinical and molecular profiling of ductal epithelium in women at risk for sporadic breast cancer, and the potential to identify important changes and components of breast carcinogenesis.
NORMAL MUCOSAL BIOLOGY AND TISSUE INVOLUTION COOPERATE TO
DRIVE BREAST CANCER METASTASIS
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Virginia Borges² and Pepper Schedin¹
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Mucosal organs serve as a protective interface against the outside environment, with promotion of barrier function and immune suppression being defining attributes. While the mammary gland is not classically considered a mucosal organ, during lactation bacteria routinely interface with luminal epithelial cells, which must serve a barrier function to prevent systemic infection. Similar barrier defenses are anticipated during weaning-induced gland involution, where milk stasis increases risk for mastitis. Postpartum breast involution is also a window of risk for breast cancer metastasis, and the link between mucosal biology and immune suppression may contribute to the poor prognosis of these cancers. One key immunologic hallmark of a mucosal organ is the presence of a distinct CD4+ helper T cell subset, Th17s. These cells stimulate epithelial cell junction integrity and secretion of mucins and defensins; mechanisms that barricade against bacterial activation of Th1 immune cells and downstream tissue destruction. Here we provide direct evidence for a Th17 immune milieu within the rodent mammary gland, which under the unique microenvironment of weaning-induced involution tips the immune balance towards immune suppression. Tumor cells within this context avoid immune detection and readily metastasize. The circulating tumor cells further benefit from weaning-induced liver involution, a previously unrecognized biology, as the involuting liver provides a tissue microenvironment highly permissive to metastatic outgrowth of circulating breast cancer cells.
ASSOCIATIONS BETWEEN REPRODUCTIVE EVENTS AND INVASIVE LOBULAR BREAST CANCER – GOING BEYOND EPIDEMIOLOGY

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Associations between reproductive events and invasive lobular breast cancer – Going beyond epidemiology

Invasive lobular breast cancer (ILBC) accounts for ~10%-15% of all breast cancers, and ranks as the 6th most common cancer in women. Despite showing better prognostic and predictive factors (e.g. more often ER+/PR+, and lower levels of the proliferation marker Ki67), there is increasing evidence that patients with ILBC have worse outcome compared to IDC. Underlying molecular reasons are unknown, but the recent comprehensive analysis of ILBC by The Cancer Genome Atlas (TCGA) has confirmed nearly universal loss of E-cadherin. Intriguingly, several published epidemiological studies have shown that the increased risk after a late age at first full time birth is stronger for patients with ILBC compared to IDC. We hypothesized that this risk is due to a dysregulation of signaling that occurs in the breast during lactation, and that this altered signaling profile might lead to a preferential development of ILC. We developed a lactation signature using mouse microarray studies, and queried the expression of these lactation genes in (TCGA) and METABRIC datasets. We found that the lactation signature is dysregulated in ILBC, as compared with IDC tumors. Many ILBC tumors show high expression of genes classically association with lactation. Additional studies need to be performed to understand whether this finding reflects a unique lineage of ILBC, and/or defects in differentiation processes, and if this can potentially be exploited for therapy of patients with ILBC.
MEASUREMENT OF SEX STEROID HORMONES IN BREAST RANDOM FINE NEEDLE ASPIRATE SAMPLES AND RELATION TO MASOOD SCORE AND GENE METHYLATION

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Background: Understanding the roles of sex steroid hormones in mammary carcinogenesis is important for breast cancer prevention and treatment, however the hormone data within benign breast tissues have been limiting. We report concentrations of sex steroid hormones in random fine needle aspirate (rFNA) of healthy breast by menopausal status and across menstrual cycle and correlations with Masood score and DNA methylation.

Methods: The breast rFNA samples from 119 healthy pre- and postmenopausal women without history of breast cancer were used to determine the concentrations of estrogens (estrone, estradiol), progesterone, and androgens (androstenedione, testosterone). Matching blood samples were used to determine the concentrations of estradiol, and progesterone. After removing triglycerides from rFNA sample, the resulting purified lipid residue was derivatized by dansyl chloride. Hormone analysis was performed for rFNA and serum samples by liquid chromatography tandem mass spectrometry and immunoassay, respectively.
<table>
<thead>
<tr>
<th>Hormones</th>
<th>Premenopausal (n=54)</th>
<th>Postmenopausal (n=65)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rFNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipid (mg)</td>
<td>1.6 (0.9,2.3)</td>
<td>4.3 (0.8,12)</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>Estrone (ng/g)</td>
<td>1.0 (0.2,5)</td>
<td>0.5 (0.1,1)</td>
<td>0.263</td>
</tr>
<tr>
<td>Estradiol (ng/g)</td>
<td>1.7 (0.8,4.3)</td>
<td>1.1 (0.4,4.7)</td>
<td>0.246</td>
</tr>
<tr>
<td>Progesterone (ng/g)</td>
<td>37 (7.43,149)</td>
<td>0 (0,0)</td>
<td><strong>&lt;0.0001</strong></td>
</tr>
<tr>
<td>Androstenedione (ng/g)</td>
<td>32 (18,49)</td>
<td>40 (25,70)</td>
<td><strong>0.026</strong></td>
</tr>
<tr>
<td>Testosterone (ng/g)</td>
<td>15 (6.2,31)</td>
<td>15 (0,40)</td>
<td>0.381</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>76 (49,141)</td>
<td>11 (0,30)</td>
<td><strong>&lt; 0.0001</strong></td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>1.9 (1.4, 5.3)</td>
<td>1.2 (1.1,1.4)</td>
<td><strong>&lt; 0.0001</strong></td>
</tr>
<tr>
<td><strong>By Menstrual phase</strong></td>
<td>Follicular (n=14)</td>
<td>Midcycle (n=19)</td>
<td>Luteal (n=21)</td>
</tr>
<tr>
<td><strong>rFNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipid (mg)</td>
<td>1.4 (0.9,2)</td>
<td>1.5 (0.9,2.6)</td>
<td>1.6 (1,2.3)</td>
</tr>
<tr>
<td>Estrone (ng/g)</td>
<td>1.2 (0.2.5)</td>
<td>1.6 (0,4.6)</td>
<td>0 (0,1.1)</td>
</tr>
<tr>
<td>Estradiol (ng/g)</td>
<td>0.9 (0.6,4.2)</td>
<td>3.1 (1.2,10)</td>
<td>1.4 (0.9,2.7)</td>
</tr>
<tr>
<td>Progesterone (ng/g)</td>
<td>11 (5.4,31)</td>
<td>7.4 (0,14)</td>
<td>151 (94,223)</td>
</tr>
<tr>
<td>Androstenedione (ng/g)</td>
<td>30 (18,35)</td>
<td>33 (18,52)</td>
<td>30 (18,54)</td>
</tr>
<tr>
<td>Testosterone (ng/g)</td>
<td>20 (13, 25)</td>
<td>6.2 (0,30)</td>
<td>15 (10,37)</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>42 (38,52)</td>
<td>174 (84,259)</td>
<td>74 (50,86)</td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>1.5 (1.4,1.9)</td>
<td>1.4 (1.2,1.7)</td>
<td>6.1 (5,9.7)</td>
</tr>
</tbody>
</table>

* Between-group comparison using Mann-Whitney U test.
‡ Multiple group comparison using Kruskal-Wallis test (ANOVA, non-parametric)
Results: We evaluated rFNA and serum hormone levels by menopausal status and across menstrual cycle (Table 1). The median total lipid amount of rFNA samples was 2.7 fold higher in postmenopausal women than in premenopausal women (p=0.003) while there was no significant difference in the lipid amounts across menstrual phases (p=0.98). The lipid amount significantly increased with age (Spearman r=0.22, p= 0.019) but did not correlate with BMI (r=0.1, p=0.278). There was no correlation between BMI and age (r= -0.001, p=0.988). Serum and rFNA levels of estradiol (Spearman r= 0.34, p=0.0002), and progesterone (r= 0.69, p<0.0001) showed strong correlations. We found that rFNA estradiol and progesterone concentrations fluctuated across menstrual cycle similar to serum hormone pattern. The rFNA estrone and estradiol levels were similar between menopausal statuses; however progesterone was rarely present in postmenopausal breast. The rFNA androstenedione concentration was significantly higher in postmenopausal women than in premenopausal women while testosterone concentrations did not change by menopausal status. The rFNA estrone (p=0.021) and testosterone levels (p=0.047) significantly changed during menstrual cycle; however androstenedione levels were relatively stable (p=0.756). The rFNA progesterone level not the serum level significantly related with Masood score (Spearman r=0.27, p=0.024) particularly with chromatin clumping (r=0.33, p=0.005). The rFNA androgens significantly correlated with cumulative methylation index (Spearman r= 0.3, p=0.002 for androstenedione; r= 0.18, p=0.049 for testosterone). At individual gene level, androstenedione correlated with ARK1B1 (p=0.004), HIN (p=0.001), and RARB (p=0.011); testosterone correlated with RASSF1A (p=0.023), and TWIST (p=0.019).

Conclusion: Quantitation of breast steroid hormones was feasible with rFNA samples. rFNA estrogen, progesterone, and testosterone levels change during menstrual cycle. It will be possible to determine menstrual phases in rFNA samples without concurrent blood collection. How breast progesterone and androgen levels affect cytopathology and gene methylation should not be ignored but may warrant a future mechanistic study.
LOCAL TRANSDERMAL THERAPY TO THE BREAST FOR BREAST CANCER PREVENTION AND DCIS THERAPY: PRECLINICAL AND CLINICAL EVALUATION
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Abstract Form

Despite successful breast cancer prevention trials that established the efficacy of selective estrogen receptor modulators and aromatase inhibitors, the acceptance of these drugs by women at high risk for breast cancer has been low. Reasons include quality of life impairments, the possibility of more serious side effects, and reluctance by healthy women to take oral medication for prevention.

Breast cancer prevention with pharmacologic agents requires that the breast be exposed to an effective drug; systemic exposure is unnecessary, and its harms lead many eligible women to decline preventive therapy. However, breast cancer prevention requires only that the breast be exposed to the drug; systemic exposure is both unnecessary and harmful. The avoidance of systemic exposure through prevention strategies that target the breast locally, and have minimal systemic toxicity, may overcome these barriers. Local transdermal therapy (LTT) to the breast involves the application of active drugs to the breast skin, resulting in high concentrations in the breast but low systemic exposure. It is non-invasive, self-delivered, and not dependent on hepatic metabolism.

Existing data on LTT include investigations demonstrating relief of mastalgia with topical 4-hydroxytamoxifen (an active tamoxifen metabolite). Two pre-surgical window trials in women with invasive breast cancer, and duct carcinoma in situ (DCIS) demonstrated that LTT decreases proliferation of invasive and non-invasive cancer cells to a similar degree as oral tamoxifen, with low systemic levels, and no effect on coagulation proteins. These data are promising regarding the use of LTT for the primary prevention of breast cancer, and for therapy of DCIS, since systemic exposure is not required for either of these purposes. They also suggest that an LTT approach could be developed for any small, lipophilic molecule with good dermal permeation, thus greatly expanding the menu of drugs that could be tested for breast cancer prevention. Therefore, local transdermal therapy (LTT) to the breast is likely to improve the tolerability and the acceptance of pharmacological cancer prevention regimens by women.

The unique features of the breast predict the success of LTT; these include the embryological origin of the breast as a skin appendage with a well-developed internal lymphatic circulation, and the presence of a subcutaneous and retromammary fatty envelope. We hypothesize that the fatty envelope of the breast may serve as drug reservoir for prolonged distribution to the breast, aided by the intramammary lymphatic circulation, so that transdermally delivered drugs are disseminated throughout the breast.
The previous data prompted further questions, 1) are other drugs candidates for LTT? 2) Is hormone receptor binding required? 3) What is the variability of drug distribution through the breast? In our recent study, we described a series of experiments to address these questions.

In a rat model, we compared two tamoxifen metabolites (4-OHT and endoxifen), and test telapristone, an anti-progestational agent. We compared the drug concentrations of mammary tissue and plasma by transdermal delivery, to those achieved by systemic therapy. Given important differences between rodent and human mammary glands, and to test a non-steroid receptor binding drug, we conducted a clinical trial to test the hypothesis that application of a transdermal diclofenac patch on the breast would result in higher breast concentrations of diclofenac than patch application to the abdomen, thus establishing that LTT to the breast constitutes local rather than systemic therapy.

Mammary tissue concentrations of 4-OHT, endoxifen, and telapristone were significantly higher in the axillary glands of the gel-treated animals, compared to inguinal glands or to systemically treated animals. Plasma concentrations were similar in gel and systemically treated animals. The clinical trial showed significantly higher mammary concentrations when diclofenac was applied to the breast skin versus the abdominal skin, but concentrations were variable. These results demonstrate that lipophilic drugs can be developed for LTT; although the nude rat is suitable for testing drug permeability, delivery is systemic. In the human however, transdermal application to the breast skin provides local delivery.

**Results:** We evaluated rFNA and serum hormone levels by menopausal status and across menstrual cycle (Table 1). The median total lipid amount of rFNA samples was 2.7 fold higher in postmenopausal women than
in premenopausal women (p=0.003) while there was no significant difference in the lipid amounts across menstrual phases (p=0.98). The lipid amount significantly increased with age (Spearman r=0.22, p= 0.019) but did not correlate with BMI(r=0.1, p=0.278). There was no correlation between BMI and age (r= -0.001, p=0.988). Serum and rFNA levels of estradiol (Spearman r= 0.34, p=0.0002), and progesterone (r= 0.69, p<0.0001) showed strong correlations. We found that rFNA estradiol and progesterone concentrations fluctuated across menstrual cycle similar to serum hormone pattern. The rFNA estrone and estradiol levels were similar between menopausal statuses; however progesterone was rarely present in postmenopausal breast. The rFNA androstenedione concentration was significantly higher in postmenopausal women than in premenopausal women while testosterone concentrations did not change by menopausal status. The rFNA estrone (p=0.021) and testosterone levels (p=0.047) significantly changed during menstrual cycle; however androstenedione levels were relatively stable (p=0.756). The rFNA progesterone level not the serum level significantly related with Masood score (Spearman r=0.27, p=0.024) particularly with chromatin clumping (r=0.33, p=0.005). The rFNA androgens significantly correlated with cumulative methylation index (Spearman r= 0.3, p=0.002 for androstenedione; r= 0.18, p=0.049 for testosterone). At individual gene level, androstenedione correlated with ARK1B1 (p=0.004), HIN (p=0.001), and RARB (p=0.011); testosterone correlated with RASSF1A (p=0.023), and TWIST (p=0.019).

**Conclusion:** Quantitation of breast steroid hormones was feasible with rFNA samples. rFNA estrogen, progesterone, and testosterone levels change during menstrual cycle. It will be possible to determine menstrual phases in rFNA samples without concurrent blood collection. How breast progesterone and androgen levels affect cytopathology and gene methylation should not be ignored but may warrant a future mechanistic study.
THE HUMAN MICROBIOME IN HEALTH AND DISEASE: AN OVERVIEW
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Abstract Form

The human microbiome refers to the multitude of microbial populations that inhabit the human body. Advances in sequencing technologies have enabled in depth characterization of these communities, most of which remain uncultivated. Different communities predominate at different body sites and these collections of microbes vary by individual. Nevertheless, community function in healthy individuals is highly conserved. Microbes perform and regulate digestion and provide essential nutrients to the host. The microbiome is responsible for educating the host immune system and commensal organisms provide barriers to prevent pathogen establishment and invasion. Microbiome studies are increasingly being applied to evaluate specific disease states. Shifts in the composition of the microbiome have been observed in diseases such as inflammatory bowel disease, asthma, allergies, autism and cancer. While in humans it is still difficult to prove causation, studies in model systems are providing compelling evidence that the microbiome is a significant component of many disease processes.
EXPOSURE TO BOVINE LEUKEMIA VIRUS LINKED TO INCREASED RISK OF BREAST CANCER
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There are numerous risk factors associated with breast cancer, but what causes the initial molecular and cellular changes from normal to malignant is not well understood. Six types of human cancer are known to be caused by viruses, and several virus candidates have been studied for their possible role in breast cancer causation. Bovine leukemia virus (BLV) is a common virus of cattle, in the USA found in 38% of beef herds, 89% of dairy herds, and 90-100% of large dairy operations (> 200 cows). Based on studies done 1970-1985 it was concluded that “BLV is not transmissible to humans and no human disease is attributed to BLV.” With the advent of more sensitive techniques/reagents (immunoblotting, monoclonal antibodies, PCR, and DNA sequencing), it was shown that humans can become BLV infected. We summarize here our three recent studies demonstrating a significant association of human exposure to BLV with the development of breast cancer.

Breast tissue specimens were formalin fixed, paraffin embedded archived tissue sections obtained from the sources listed in Table 1. In situ PCR was performed on intact deparaffinized sections on glass slides, using primers from the \textit{tax} region (transforming gene). Sections were semi-quantitatively scored for density of the PCR product within mammary epithelial cells upon microscopic examination by two independent examiners.
BLV-positive lactating bovine mammary gland tissue with and without primers in the PCR mix. Dark staining = positive cells, some surrounding milk-filled lumens. No reaction in adjacent section of tissue (right) means no false positive background. 

Human tissue sample with BLV-positive epithelial cells facing the lumen of a large cyst. Reaction is primarily cytoplasmic.

BLV-negative human tissue sample.

Photo from Emerging Infectious Diseases 20:772-782, 2014

Table 1. Summary of results of three case-control studies examining the relationship of exposure to BLV (indicated by presence of PCR-amplified BLV DNA in mammary epithelial cells) to a disease outcome of breast cancer (indicated by a pathology-confirmed diagnosis):

<table>
<thead>
<tr>
<th>Source of specimens</th>
<th>Sample number</th>
<th>Frequency of BLV-positive samples</th>
<th>Odds ratio (95% confidence interval)</th>
<th>Probability of happening by chance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooperative Human Tissue Network (CHTN, Southern and Eastern Division)(^1)</td>
<td>n = 239</td>
<td>30/104 (29%) Pre-malignant</td>
<td>67/114 (59%)</td>
<td>3.07 (1.66 – 5.69)</td>
</tr>
<tr>
<td>Douglass, Hanley, Moir Pathology, Macquerie Park, NSW, Australia(^2)</td>
<td>n=96</td>
<td>19/46 (41%) -----</td>
<td>40/50 (80%)</td>
<td>4.72 (1.71-13.05)</td>
</tr>
<tr>
<td>MD Anderson Cancer Center, Houston, TX(^3)</td>
<td>n = 216</td>
<td>20/105 (19%) Pre-malignant</td>
<td>35/61 (57%)</td>
<td>5.59 (2.76-11.30)</td>
</tr>
</tbody>
</table>

\(^1\)PLoS ONE 10(9): e0134304, 2015. \(^2\)Emerging Infectious Disease (manuscript submitted), \(^3\)(manuscript in preparation)

For 48 of the Australian subjects with breast cancer, an archived normal breast tissue was available from breast surgery performed 3-10 years previously for a condition unrelated to the subsequent malignancy. For 23/31 (74%) of these, BLV was already present in the normal tissue at least 3 years before a diagnosis of cancer, consistent with a causative temporal relationship between BLV infection and the subsequent development of cancer.
An unexpected outcome occurred in the two studies in which there were African-American subjects. The overall frequency of BLV DNA in mammary epithelial cells was significantly less in women of African (29%) versus Caucasian ancestry (50%), and was not related to breast cancer status, consistent with the idea that the primary initiating agent for breast cancer in African-American women may be something other than BLV.

Discussion:
One strength of this research is the selection of primers from the tax region of the BLV genome, which codes for the gene responsible for the malignant transformation of cells. Its sequence is highly conserved and rarely deleted, as happens with most other regions of the BLV genome as tumor progression occurs. Specificity of the tax primer pair was confirmed by NCBI GenBank (nucleotide BLAST) sequence comparisons to have the highest homology with BLV, and using comparison with human genomic sequences, including human endogenous retroviral sequences, a very low homology. No cross-reactivity of the primers occurred in laboratory experiments with representatives of all oncogenic retroviral families and HERV-K (human endogenous retrovirus).

Another strength of this research is the technique of in situ PCR, advantages of which are 1) DNA is not digested and the signal can be located to the cell type in which target DNA is present; 2) the reaction cannot be contaminated with extraneous DNA as with standard solution PCR. A disadvantage of in situ PCR is that it requires sample preparation, cycling times/temperatures, reaction mix composition, and thermal cycler equipment different from those used for standard solution and real-time PCR, thus necessitating an investment of time and practice to achieve accurate results, but the rewards are well worth it.

The results of these case-control studies have significant potential application in clinical practice and public health. One primary mechanism of malignant transformation of BLV is inhibition of DNA repair, which allows accumulation of multiple random mutations in the genomes of infected cells, exactly what investigators have observed and mapped in breast cancer cells. At this point, how humans become infected with BLV is not known. It could be directly from bovine foodstuffs or from other humans already infected. If BLV is transmitted from cattle, primary prevention strategies could be instituted. BLV could be eradicated from herds to prevent further transmission. Veterinary spokespeople are strongly recommending this to the agricultural industry, based on our published article. A bovine vaccine could also be developed, and perhaps modified for human use. The dangers of human consumption of raw milk and raw meat could be publicized more extensively (BLV is inactivated by pasteurization of milk and thorough cooking of beef). In the event human-human transmission occurs, one likely route of transmission may be mother to child in breast milk, as this is the main route of BLV transmission in cattle and for human T-cell leukemia virus, a close relative of BLV. Breast milk transmission could be intercepted by pasteurization of breast milk or cessation of nursing after 6 months when the protective maternal antibody level has declined. Secondary prevention might also be fruitful. Further research could lead to streamlined diagnostic methods that could reveal the presence of BLV in breast tissue and allow the possibility of surgical or pharmaceutical intervention before the tissue has progressed to malignancy.
ASSOCIATIONS OF THE GUT MICROBIOTA AND SYSTEMATIC ESTROGENS WITH BREAST DENSITY AND BREAST CANCER AMONG POSTMENOPAUSAL WOMEN
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Abstract Form

Background: The population of microbes (the microbiota) in the distal gut affects systemic levels of estrogens through enterohepatic cycling and probably other mechanisms. High systemic levels of estrogens, and especially high breast density, put postmenopausal women at increased risk for breast cancer.

Objective: For new insights on these associations, at Kaiser Permanente in Colorado we investigated whether systemic estrogen levels or the gut microbiota were associated with breast density in randomly selected, postmenopausal women, and whether estrogen levels or the microbiota differed between these healthy controls and 48 postmenopausal breast cancer cases (two stage 3, ten stage 2, 25 stage 1, 11 in situ), pre-treatment.

Methods: We classified low- versus high-density controls with Breast Imaging Reporting and Data System (BI-RADS, 5th edition) mammographic screening data. Estrone, estradiol, and 13 estrogen metabolites in urine were quantified by liquid chromatography/tandem mass spectrometry. Microbiota profiles in fecal DNA were determined by Illumina sequencing and taxonomy of 16S rRNA genes. High- versus low-density and case-control comparisons employed linear and unconditional logistic regression of microbiota α-diversity (PD_whole tree) and UniFrac and MiRKAT analyses of β-diversity, with two-sided statistical tests. Analyses were adjusted for age (mean 62) and body mass index (BMI, mean 28).

Results: Adjusted for age and BMI, breast density among controls was strongly and inversely associated with estrone, estradiol, all 13 metabolites, and the total of these (P=0.01). Breast density among controls was unrelated to fecal microbiota metrics (P≥0.82). Compared to controls, cases had two-fold, non-significantly higher total estrogens (45 vs 22 picomole/mg creatinine, P=0.12), and lower microbiota α-diversity (P=0.004). Adjusted for estrogens, age and BMI, odds ratio of cancer was 0.50 (95% confidence interval =0.30–0.85) per α-diversity tertile. Total estrogens correlated with α-diversity in controls (Spearman Rho=0.37, P=0.009) but not cases (Rho=0.04, P=0.77). Compared to controls, cases also had significantly altered microbiota composition (β-diversity, P=0.006), but differences in specific taxa were not statistically significant when adjusted for multiple comparisons.

Conclusion: This investigation found that postmenopausal women with breast cancer had altered composition and estrogen-independent low diversity of their gut microbiota. It also
found that mammographic density in postmenopausal controls was not associated with their
gut microbiota, but paradoxically it was inversely associated with urinary estrogen levels. These
observations suggest that cancer may arise in a small subset of postmenopausal women who
have high estrogens, high breast density, and low diversity plus altered composition of their gut
microbiota. Prospective studies are needed to test this hypothesis.
Recent reports of remarkable results in treatment of hematological malignancies such as chronic lymphocytic leukemia, using genetically engineered autologous T cells called CAR T cells (chimeric antigen receptor T cells), has generated great deal of interest in developing similarly effective approaches in treatment of solid tumors. In order to advance these therapies into the world of solid tumor types, we must gain a better understanding of the role T cells play in antitumor immunity and immune surveillance. There is still a dearth of information on the role of T cells in human breast and breast cancer to allow immune therapies to have the success in breast cancer as in other tumors. One of the limiting factors in studying breast T cells is the availability of sufficient biopsy tissue from the excision of primary tumors. In this study using a 3-D tissue explant approach, previously described for skin T cells, we expanded tumor infiltrating T cells from fresh breast cancer tissue and examined phenotypic and functional qualities of breast T cells using multiparameter flow-cytometry and multiplex Luminex assay. Examination of the expanded breast tumor infiltrating T cells showed presence of both CD4 and CD8 T cells with CD4 T cells at slightly higher frequency (means CD4 54% vs. CD8 35%, p=0.07). Analysis of memory populations utilizing expression of the memory markers CD45RO and CD62L revealed the dominant phenotype to be the effector memory (EM) subtype (CD45RO+, CD62L-) in both CD4 and CD8 compartments (means CD4 69% and CD8 65%). When samples were grouped by clinical information, we found that the T cell profile from breast tissue of breast cancer patients showed a distinct T cell profile associated with regional lymph node progression (LN+) vs. disease localized to the primary site (LN-). We found that the frequency of effector type CD8 T cells (EFF) (CD45RO-CD62L- subtype) was higher in breast tumor tissue of patients without lymph node metastasis (LN-) than those with metastasis (LN+) (means 32% vs 12%, p= 0.01). Similar pattern was observed in CD4 compartment, although the overall frequency of CD4 EFF cells was much lower than CD8 (means 5% vs 1%, p= 0.04). Analyses of culture supernatant of the expanded T cells via multiplex Luminex assay showed robust production of cytolytic granule proteins at basal state. Levels of granzymes A and B were significantly higher in culture supernatants of breast tumor infiltrating lymphocytes of LN-patients compared to LN+ patients (3815 vs 1645 pg/mL, p=0.005, 500 vs 268 pg/mL, p=0.04, respectively) while there was no difference in the levels of perforin. More importantly the frequencies of total CD4 or CD8 T cells were not significantly different in breast tumor tissue of
LN- vs LN+ patients (means CD4 57% vs. 51%, p=0.6, CD8 30% vs. 40%, p=0.3). Lymph node status is the single most important indicator of disease-free survival and overall survival in breast cancer. Our observation of a distinct T cell profile associated with lymph node metastasis strongly suggests that our 3-D explant method of T cell expansion could serve as an important profiling / prognostic tool. Furthermore, our finding of higher levels of granzymes associated with LN- disease status begs consideration of including these molecules during selection of T cells for adoptive T cell therapy in breast cancer.
Breast cancer is the leading cause of cancer death in women worldwide and despite advances in diagnostics and treatment strategies, the World Health Organization predicts that the number of deaths will continue to rise over the next 20 years. The etiology of breast cancer is still unknown, but thought to be due to a combination of both genetic and environmental factors. While many environment predictors have been proposed one not yet considered is the role of the host’s microbiome, specifically the breast microbiome, in breast cancer development or prevention. The aim of this study was therefore, to assess microbial profiles in breast tissue from women with and without breast cancer and to examine the consequences of an altered bacterial community in terms of breast cancer development. To investigate this, we collected breast tissue samples from a total of 120 women from both Canada and Ireland who had either breast cancer, benign tumors, or who were disease free.

Using next generation sequencing we have shown that breast tissue is not sterile as originally believed but contains a diverse population of bacteria. Differences in bacterial profiles were observed between healthy individuals (i.e. those going in for breast reductions and enhancements) and normal adjacent tissue of women with breast cancer or benign tumors. Women with breast cancer had higher proportions of bacteria that could cause DNA damage and the potential to regulate hormone metabolism. A metabolomics analysis revealed different breast tissue metabolites between breast cancer and healthy individuals, with certain metabolites implicated in either development or protection. Some of these metabolites could also be correlated back to the differentially expressed microbes in the different cohorts.

This study raises important questions as to the role of the breast tissue microbiota in breast cancer development or protection and whether bacteria could be harnessed for interventions to help prevent disease onset.
VARIATIONS IN THE BREAST TISSUE MICROBIOME WITH DISEASE STATES AND BREAST CANCER RISK FACTORS
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Abstract Form

The complex microenvironment of breast tissue includes epithelium, stroma and a mucosal immune system providing evidence for an intrinsic breast tissue microbiome. Our recently published data using culture-independent genomic analysis of sterile human breast tissue confirms the existence of a breast tissue microbiome, distinct from other niches. We assessed breast tissue microbial signatures in intraoperatively obtained samples using 16SrDNA hypervariable tag sequencing, along with simultaneously aseptically collected skin tissue and skin swab samples. Our results indicate a distinct breast tissue microbiome different from the microbiota of skin tissue, surface breast skin swabs and buccal swabs. Further, we found distinct microbial communities in breast tissues from women with breast cancer vs women with benign disease. Malignancy correlated with enrichment in taxa of lower abundance including the genera *Fusobacterium*, *Atopobium*, *Gluconacetobacter*, *Hydrogenophaga* and *Lactobacillus*. Testing for confounding showed no significant effect of age and menopausal status (MiRKAT P>0.05) indicating the differences were not driven by these factors. *Fusobacterium* is reported in other epithelial malignancies and may act by secreting virulence factors as well as creating a pro-inflammatory environment promoting carcinogenesis. Therefore, we investigated the functional role of the breast tissue microbiome within these microenvironments. Using KEGG pathways, we identified 6 as differentially abundant between benign vs malignant disease states. In patients with breast cancer, pathways involving cysteine and methionine metabolism, glycosyltransferases, fatty acid biosynthesis and C5-branched dibasic acid metabolism were depleted.

Mammographic breast density (MBD) is a well-established breast cancer risk factor conferring a 2 to 4-fold elevated risk in women with heterogeneously or extremely dense breasts. As bacterial communities influence immune-mediated inflammatory changes that affect stroma in ways that may affect breast carcinogenesis, we further explored how microbial composition within breast tissue might correlate with MBD. For BI-RADS density defined as high or low we saw a trend toward lower alpha diversity in high-MBD samples (linear regression P=0.13 for observed OTU number, P=0.23 for Shannon index). Beta-diversity analysis showed a significant association on weighted (MiRKAT P=0.049) but not unweighted UniFrac distance (MiRKAT P=0.31), indicating a MBD association with community composition (taxa abundance) rather
than structure (taxa presence or absence). Boruta feature selection identified two genera from the phylum *Actinobacteria*, *Corynebacterium* and *Actinomyces*, as most predictive of MBD suggesting a microbiome component of MBD-associated breast cancer risk.

While it is unknown whether overall microbial community shifts, presence of a dominant virulent pathogenic strain or absence of a beneficial one might contribute to breast carcinogenesis, our data generate interesting hypotheses and support further investigation to identify a microbial risk signature for breast cancer and potential microbial-based prevention therapies.
While a huge effort has emerged to define the human microbiome in the gut, skin, and other organ systems, there has been less attention on tissues previously thought of as sterile, including breast tissue. A few studies have defined the bacterial microbiome in human breast milk and breast tissue, and the physiologic role of bacteria or their components in breast cancer has not been well defined. The presence of bacteria at the site of the tumor microenvironment may contribute to carcinogenesis (e.g., by promoting chronic inflammation) or immune surveillance (e.g., by stimulating antitumor pathways). Our investigation of the microbiome of breast tissue and potential implications will be discussed.
Abstract Form

The health of most living organisms depends critically on the transport of bio-fluids within and between organs. In particular, any disruption or deficiency in bio-fluid transport can result in various diseases in the human body. Therefore, the understanding of these bio-transport phenomena has a crucial role in the advancement of science as well as bio-engineering. Bio-transport phenomena have been extensively studied in the lung, heart, and nasal airways, among others. These studies have found relevance in the diagnosis and treatment of diseases as well as the development of various bio-medical devices and surgical procedures. The female human breast is a prime example of the principle mentioned above. Many of the diseases of this organ are closely related to the ductal system transporting fluids; among these diseases one can name breast carcinoma, ductal blockage, breast engorgement, breast abscess, and galactocele. Despite the scientific importance and impact on public health, bio-transport processes in the human breast have not been comprehensively studied, and are currently not well-understood. The better understanding of transport processes in the breast can potentially lead to many advances in the diagnosis and treatment of the pathologies mentioned above, with a huge societal impact. Our studies focus on scientific questions that have a direct bearing on the fundamental understanding of milk flow transport processes. This research includes three components of mathematical investigation, Computational Fluid Dynamics (CFD) modeling, and experimental analysis.

Mathematical Investigation: Milk flow in the lactating breast has two paths: diffusion of blood-derived components through alveoli (diffusion resistance), and flow through mammary ducts (convective resistance). Flow resistance is an important mechanical property of the breast ductal system that must be successfully overcome by the sucking pressure. The total flow resistance is minimized when the structure is arranged in a way that the flows with high resistance (diffusion in alveoli) occupy the smallest scales of the flow system, while the flows with the lower resistance (convection in ducts) inhabit the larger scales. Our mathematical model uses this principle in the branching structure of human breast to arrive at predictions that are verified by their close match with several clinically measured quantities. More specifically, we arrive at preliminary formulas that predict parameters such as alveoli dimension that vary from subject to subject, are clinically important, and are difficult and expensive to measure (requires biopsy). Our formulas calculate estimates of these important internal parameters using easily measurable parameters such as milk flow properties and
(external) dimensions, e.g. diameter of nipple ducts, therefore the formulas have an immediate practical impact. The prediction accuracy of these formulas has been cross-validated with biopsy samples of alveoli via microscopic imaging.

**Computational Fluid Dynamics Modeling:** Numerical analysis of the flow inside the breast provides a unique understanding of the related internal processes. In particular, the results from a CFD study can reveal flow behavior, such as pressure and velocity profiles inside each of the ducts, and their relationship with the geometry of the ductal system for an individual subject, as well as the related boundary conditions (infant sucking). This knowledge is at the present time impossible to obtain with medical or surgical procedures. One of our research tasks is a 3D Computational Fluid Dynamics modeling of milk flow in lactating human breast. This collaborative effort among lactation specialists and fluid dynamic engineers represents measurement of suckling patterns, milk flow rate and milk intake on a group of infants. The clinical data are then used as the boundary condition for the CFD study using commercial ANSYS FLUENT software. For the geometric model of the ductal system of the human breast, this work takes advantage of a recent advance in the development of a validated phantom that has been produced as a “ground truth” for the imaging applications for the breast. The geometric model is introduced into CFD simulations with the aforementioned boundary conditions. The results for milk intake from CFD simulation and clinical data are compared and cross validated. Also, the variation of milk intake versus suckling pressure are presented and analyzed.

**Experimental Analysis:** CFD modeling of the breastfeeding mechanisms requires obtaining a number of complex parameters such as the physical forces exerted by the infant during breastfeeding and the flow properties of human milk. While there are several studies in literature about the patterns and values of vacuum pressure (negative pressure) exerted on the nipple, the positive oral pressure exerted by the infant’s mouth on the nipple-areola complex during breastfeeding has received limited attention. In this study, ultrasound images from cross section of the oral cavity and time dependent motion of the wave-like tongue movement during breastfeeding are extracted by an endocavity convex transducer placed under infant chin. This imaging takes place in parallel to the pressure data collection. Pressure data are obtained using the Tekscan™ I-Scan System flexible resistance pressure sensor strips (for mouth pressure on the breast surface) simultaneously with a tubing pressure transducer (for the vacuum pressure). Through these experimentations, we successfully captured pressure exerted on the breast from an infant’s upper and lower lips/gums and revealed their correlation with the vacuum pressure and milk ejection. These data are being used to modify the boundary conditions for simulations of breastfeeding and provide improved understanding of the mechanics involved in milk extraction from the breast. Additionally, there are several studies in literature concerning the chemical composition of human milk but very little work has been done on the flow properties of milk in the human breast ductal system. In this study, raw human milk was tested for variations in viscosity in response to changing shear rates and temperatures using an Anton Paar™ MCR 302 rheometer. These data are being used in combination with the pressure data to simulate the flow behavior of milk from alveoli to ejection and understand the origin of milk stasis and effectiveness of current treatments to reverse milk stasis.
APPLYING AUTOMATED BREAST ULTRASOUND AND COMPUTER AIDED DETECTION FOR THE
GENERATION OF THE NORMAL BREAST’S DUCTAL PATTERN:
WHAT WE LEARNED FROM A PILOT STUDY

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Abstract Form

Purpose: The long-term purpose of our work is the development of a 3D map of the healthy
breast ducts through 3D imaging and computer processing algorithms. In this first study, we
evaluated the ability of the 3D automated breast ultrasound systems to detect the breast ducts
by a pilot imaging study with 6 lactating women.

Materials & Methods: Six lactating, nursing volunteers were scanned with the Invenia™
Automated Breast Ultrasound (ABUS) system (GE Healthcare, WI, USA). Mean age was 33.5
years and mean lactation time was 10.5 months; three of the women were primipara and three
were multipara. The women were imaged before and right after breastfeeding their infants.
Three image volumes were recorded for each breast, an anterior-posterior (AP) volume, a
lateral (LAT), and a medial (MED) volume. An expert in breast ultrasound generated gold
standard files of the milk-filled ducts by outlining all such areas in the AP volume slices using
ImageJ. In addition, all image volumes were processed with a JPL computer aided detection
(CAD) algorithm that included a noise reduction step, a segmentation step based on localized
means and standard deviations, and a volume rendering step where all three image volumes
were used to determine pixel association and delineate the final ductal patterns. CAD results
were compared to the expert’s gold standard files by estimating true positive (% overlap), true
negative, and false negative fractions. Breast and duct/milk volumes were also estimated and
compared to the literature.

Results: Table I shows the volumes of milk-filled ducts identified by CAD and the expert in the
AP images of the ABUS per breast side and condition (pre- and post-nursing). Based on the AP
images only, the average right breast volume was 606.9 ml (SD of 233.9 ml) and average left
volume was 631.5 ml (SD of 248.8 ml).
Table I. Duct volumes per breast, pre- and post-nursing, as detected by CAD and the expert.

<table>
<thead>
<tr>
<th>Volunteer #</th>
<th>RIGHT BREAST DUCT VOLUME (ml)</th>
<th>LEFT BREAST DUCT VOLUME (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE-NURSING</td>
<td>POST-NURSING</td>
</tr>
<tr>
<td></td>
<td>CAD EXPERT</td>
<td>CAD EXPERT</td>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
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<tr>
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</tr>
<tr>
<td>4</td>
<td>1.25</td>
<td>na</td>
</tr>
<tr>
<td>5</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>2.71</td>
<td>0.22</td>
</tr>
</tbody>
</table>

The detected duct volumes were small compared to the entire breast area (maximum up to 1%) showing that only a small part of the ductal system was detectable since only parts of some of the ducts were filled with milk and, consequently, only parts of some of the ducts demonstrated sufficient contrast to be observed with the ABUS. Based on the expert’s results, pre-nursing, the left breast shows more milk-filled ducts than the right breast in 5 of the 6 volunteers (difference is statistically significant with P=0.046). Post-nursing, the left duct volume is significantly reduced but the change is not as significant for the right breast leading to non-statistically significant differences (P=0.45). An analysis of the upper and lower halves of each breast showed that more milk-filled ducts were detected in the lower half than the upper half in 5 out of 6 volunteers (Table II); these results agree with earlier studies on ductal imaging with hand-held or 3D ultrasound systems and seem to be linked to lactation times. However, our sample is too small to reach definitive conclusions.

Table II. Pre-nursing duct volumes per breast side and half (lower and upper) as detected by the expert.

<table>
<thead>
<tr>
<th>Volunteer #</th>
<th>RIGHT BREAST DUCT VOLUME (ml)</th>
<th>LEFT BREAST DUCT VOLUME (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOWER HALF</td>
<td>UPPER HALF</td>
</tr>
<tr>
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<td>1.40</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>0.55</td>
<td>0.39</td>
</tr>
<tr>
<td>3</td>
<td>0.78</td>
<td>0.86</td>
</tr>
<tr>
<td>4</td>
<td>0.87</td>
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</tr>
<tr>
<td>5</td>
<td>0.96</td>
<td>0.32</td>
</tr>
<tr>
<td>6</td>
<td>0.17</td>
<td>0.05</td>
</tr>
</tbody>
</table>

CAD results differ significantly from the expert’s. The true positive fraction (TPF) of CAD (or % overlap with the expert’s gold standard) ranged from 10% to 39% for the left breast and from 0.1% to 34% for the right breast. The relatively low performance compared to the gold standard seems to be a direct result of the empirical selection of the thresholds at the various processing stages of the initial algorithm.

Conclusions: In this pilot study, we attempted to detect and outline the breast ductal pattern in lactating women using a 3D ABUS and a CAD methodology. Results allowed us to identify the weaknesses and strengths of this approach for the specific task. The advantages of the methodology were: (a) the non-ionizing radiation, (b) the low cost of the procedure and
convenience of use, (c) the relatively low imaging time, and (d) sufficient resolution for the
detection of ducts assuming a contrast enhancing mechanism exists, e.g., milk-filled or saline-
filled ducts. The limitations of the approach included: (a) Insufficiently optimized ABUS imaging
protocol for the specific application, which requires appropriate compression to avoid the
collapse of the ducts while providing sufficient contrast. (b) Breast ultrasound expert needs a
parallel training, i.e., training with handheld systems and the new ABUS, if complete and
reliable gold standard files are to be generated. (c) CAD could provide accurate duct outlines
from all three volumes, AP, LAT, and MED. CAD could also correct discontinuities and merge
parts of the same duct together so that the complete 3D structure is generated. However, more
sophisticated CAD algorithms are required that will match the ABUS characteristics and
increase TPF; pilot data could guide modifications and provide a training set for optimization
procedures. Finally, (d) the lack of an appropriate phantom for such studies hinders
optimization and evaluation of both the imaging protocol and the CAD performance.
3D MODELING OF BREAST TISSUE SUBTYPES USING MRI DATA OF CADAVERIC BREAST TISSUE WITH INTRADUCTAL CONTRACT INJECTION

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Abstract Form

Introduction: In creating a 3D model of the breast tissue types, the purpose of this study is to contribute to the available knowledge on ductal anatomy to support research on targeted therapies for ductal carcinoma in situ (DCIS). Additionally, the study is a proof-of-concept of the use of MRI sequencing and Amira software to generate a 3D model of breast ducts, fat, and fibroglandular tissue. DCIS is the most common non-invasive lesion of the breast. It can be a vexing problem in breast oncology because physicians often cannot predict if, or when, it may become invasive and thus it can be difficult to advise patients on appropriate treatment options. Some women chose to have prophylactic mastectomies due to lack of more targeted therapies for DCIS. Improvements in targeted therapies require a better understanding of the breast anatomy – specifically the duct network. Additional models of duct anatomy based on human tissue samples are needed in this effort, as a reliance on idealized illustrations are not sufficient in illuminating patient-to-patient differences as well as imperfect distributions of ducts within the volume of a breast.

Methods: Dissections of two cadaveric breasts from the same cadaver were provided through UCSD School of Medicine’s Body Donor Program. The breast tissue underwent baseline imaging and then was injected with gadolinium contrast through the visible duct openings at the nipple. After MRI data was collected, it was uploaded into Amira – a 3D modeling software – where individual tissue types (fat, fibroglandular tissue, ducts) were segmented and rendered individually and three-dimensionally.

Results: The MRI data was successfully segmented into three types of breast tissue – fat, fibroglandular tissue, and ducts – using Amira. Therefore, this is a proof-of-concept for the use of MRI data and Amira to obtain 3D images of breast tissue subtypes, which can be applied to future studies. Please refer to figures 1 through 10 for images of the 3D renderings.
Conclusions: The 3D computer models created in this study contribute to the available knowledge of breast anatomy and importantly, duct anatomy. Such contributions have been limited since Cooper’s 1840 wax model. A better understanding of the duct anatomy is important in the advancement of treatment for DCIS in the hopes of avoiding unnecessary mastectomies while also preventing the development of invasive breast cancer after a DCIS diagnosis.

Fig. 1 and 2: Isolated Ducts

Fig. 3, 4, 5: Isolated Fat

Fig. 6, 7, 8: Ducts Within Fat
Fig. 9, 10: Isolated Fibroglandular Tissue

Images of 3D Prints of Fat
References:


The heterogeneity of breast cancer poses a formidable challenge for effective treatment, risk management, and disease prevention. Presently, three predictive biomarkers with cognate therapies (ER, PR, HER2) are in widespread clinical use, and while they have improved treatment options and disease outcome, there remains a substantial number of patients who either do not respond to treatment or whose tumors do not express any of these biomarkers (i.e. “triple negative” breast cancer; TNBC). Recent advances in genomics have started to make headway into breast cancer heterogeneity; however, the molecular diversity of breast tumors, even within the more refined ‘intrinsic’ subtypes, confounds discovery of urgently needed new biomarkers and drug targets. In addressing the problem of breast tumor diversity, we have identified another significant source of heterogeneity: left-right (L-R) positional identity. We have recently reported that despite their identical appearance and function, the left and right breasts have unique developmental histories, including patterning of the stromal compartment. We hypothesize that one consequence of L-R differences in the stromal microenvironment is to promote lateralized differences in tumor biology when neoplasia occurs. To test this, we evaluated effects of the left versus right mammary microenvironment on tumor growth and progression using the syngeneic 4T1 TNBC mouse model. Luciferase-expressing 4T1 metastatic carcinoma cells were injected into either left or right mammary glands of adult female mice. Cell viability and tumor take were confirmed by imaging luciferase activity. Upon sacrifice, there were L-R differences in the number of mice showing macrometastatic lung lesions (33% left group versus 83% right group; N=12, P< 0.05). Assessment of tumor volume by caliper measurement, IVIS luciferase imaging, and endpoint tumor mass indicated that attenuated metastasis of left tumors was not due to slower tumor growth. Moreover, analysis of micrometastases indicated no significant differences in dissemination of left or right tumor cells to the lung, suggesting that differences in metastasis reside in differential colonization of the metastatic niche. To determine if left-side tumors remain indolent, experiments were repeated with later endpoint analysis. We found that most left-side tumors eventually do metastasize with incidence comparable to right-side tumors (83% left group versus 100% right group; N=12, P=0.24), but the size and number of lung nodules nevertheless were decreased compared to those arising from right-side tumors. To determine if L-R differences are present in TNBC patients, we compared transcriptomes of left (N=55) versus right (N=54) TNBC tumors. Preliminary findings, based on >2.5-fold L-R differential
expression with $P<0.05$, have yielded distinct left versus right human TNBC signatures. Studies
are in progress to refine these signatures and to identify the pathway(s) that promote L-R
differences in tumor cell-microenvironmental interactions. Elucidation of these pathways is
anticipated to reveal mechanistic information on TNBC metastasis and in turn, to identify
candidate biomarkers and therapeutic targets that likely otherwise would be missed in L-R
aggregate analyses.
Ductal Carcinoma In Situ (DCIS), the most frequently diagnosed subgroup of new breast cancer cases, has several molecular subtypes including luminal A (ER+ and/or PR+, HER2-, Ki67 <14%), luminal B/HER2- (ER+ and/or PR+, HER2-, Ki67 ≥14%) or luminal B/HER2+ (ER+ and/or PR+, HER2+), HER2+/ER- (ER-, PR-, HER2+), and triple negative (ER-, PR- and HER2-). Triple negative DCIS has an elevated risk of recurrence. In addition, cancer stem cells are considered to play critical roles in both metastasis and local recurrence of malignancy. Thus, the diversity of DCIS presents a challenge for its treatment. Our group is targeting DCIS subtype specific, as well as commonly expressed, molecular markers (including cancer stem cell antigens) to develop intraductal drug delivery systems (DDSs).

Novel strategies for targeting DCIS: Previous approaches for targeting DCIS have focused on using only passive (intraductal) targeting of cancer cells to either enhance ductal retention or prolong local drug concentrations. The identification of molecular markers for DCIS has led us to utilize active targeting to potentially further increase the effectiveness of DDS. Thus, in our current research, we aim to use a novel strategy that involves both passive and active targeting of DCIS—at both the cellular and tissue level. A well-defined cancer marker, the transferrin receptor (TfR) is overexpressed in breast cancer cells (including the DCIS type), but not in normal mammary duct epithelium. CXCR4 and HER2 receptors are often overexpressed in higher grade (more invasive) subtypes of DCIS. CD44 is a well-established biomarker for breast cancer stem cells. Therefore, by dual or multimodal targeting, it is possible to treat all subtypes of DCIS. Moreover, HER2, CXCR4 and CD44 targeting provide precise delivery against the more aggressive subtypes. Furthermore, combining these targeting tools produces a synergy, providing more personalized therapeutic delivery strategies.

Ligands for active targeting: In active targeting, ligands with specific binding ability are selected to deliver therapeutic agents. Such targeting moieties may include: TfR binding peptides (e.g. TfRBP9), HER2 antibodies or binding peptides (e.g. Herceptin, Pep27), and CXCR4 binding peptides (e.g. T140, DV3 and Peptide R). 4DV3, a CXCR4-specific ligand developed in our laboratory, consists of 4 copies of an optimized 10AA long D-peptide. We found it could deliver streptavidin into TZM-BL cells in a CXCR4-dependent manner, demonstrating its targeting
capability for nano-sized drug carriers. Lastly, CD44 ligands include hyaluronic acid and certain CD44 binding peptides.

**Therapeutic agents:** We found that ciclopirox (CPX), an anti-fungal drug, possesses promising anti-cancer effects. It displays strong cytotoxicity in MCF10DCIS.com, MCF7 and MDA-MB-231 cell lines, as well as inhibition of mammosphere formation. CPX nanosuspensions showed complete inhibition of tumor growth in an *in vivo* DCIS orthotopic rat model. We also tested polyethylene glycol-doxorubicin (PEG-DOX) conjugates *in vivo*, and observed significant anti-cancer activity in rats.

**Conclusion:** The use of passive targeting combined with novel active targeting strategies promises to be a powerful therapeutic modality, affording a more personalized approach for DCIS treatment.
FOCUSED MICROWAVE THERMAL THERAPY SYSTEM WITH INTEGRATED REAL-TIME MONITORING
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Abstract Form

In the current standard-of-care treatments for breast cancer (surgery, chemotherapy, hormonal therapy, and radiation therapy), there remains a need for the reduction of local recurrence, harmful side-effects, and cosmetic harm. Toward that end, we have investigated a microwave thermal therapy and monitoring system for the targeted treatment of breast cancer cells using focused microwaves as an adjuvant to radiation, chemotherapy, and high intensity focused ultrasound (HIFU). This talk summarizes the current state of our work.

An array of antennas operating at 915 MHz is used to focus continuous-wave (CW) microwave energy transcutaneously into the pendent breast suspended in a coupling medium. Prior imaging studies are used to ascertain the material properties of the breast tissue. These data are incorporated into a multiphysics computational model to enable adjustments to the relative amplitude and phase for each antenna array element for focusing at a given location and to optimize treatment planning for ablation at the tumor location while minimizing the thermal dose elsewhere in the breast. A set of custom-designed RF modules, each with individual digital control, is then used to deliver up to 120 W of total microwave power (CW) to the antenna array. Using this array, the microwave therapy system is able to achieve focal spots of approximately 2 cm anywhere within the breast.

In addition, to provide real-time feedback and control during thermal therapy, we have developed a method for remote non-contact mapping of temperature fields using an integrated active microwave imager. The imaging array can be built into the same apparatus as the therapy array, and may also be used for monitoring of other thermal therapy modalities.
RAPID CANCER DIAGNOSIS IN THE REMOTE AREAS OF THE WORLD USING AN AUTOMATED GENE TEST

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Background: In 2012 underdeveloped countries reported 882,900 new cases of breast cancer and 324,000 deaths, a mortality level similar to the developed world even though the lower detection incidence. In these countries, population-based mammography screening is not yet available, primary care services are limited, and pathology and treatment services are nascent and rudimentary. Women in these regions generally present with a large palpable mass as a first indication of a problem and the challenge is to distinguish benign breast disease versus cancer in a timely manner. Our goal is to provide timely diagnostic testing to underserved women as an ancillary service to local pathological diagnostic practices.

Preliminary data: We have developed a cancer-diagnostic assay of DNA methylation in tissue called Quantitative Multiplex Methylation-Specific PCR (QM-MSP). Methylated tumor-specific DNA can be sensitively and specifically detected in samples such as ductal lavage and ductoscopy fluids, fine needle aspiration, core biopsy in a background of 100,000 normal cells.

Results: Here our goal was to develop a panel of methylated gene markers that distinguishes between benign breast disease and invasive cancer with high sensitivity and specificity. Available were 24 methylated gene markers we previously developed in our laboratory that are high in frequency and intensity in cancer and low in normal tissue. These were tested by QM-MSP in a Training set consisting of 93 patient samples [n = 43 invasive ductal carcinoma (IDC) and n = 50 benign (25 usual ductal hyperplasia, UDH, and 25 papilloma)]. Among the candidate markers we observed 9 genes significantly (p < 0.05; Mann-Whitney U Test) more methylated in malignant breast cancer compared to benign breast disease. The 9 markers were then tested as a panel on a Test set consisting of benign (n = 26) and malignant (n = 10) breast FFPE tissues. When these markers were analyzed in the Test set as a locked panel, the sensitivity was 90% and specificity was 92%, with a laboratory cutoff of 9 CMI units (out of a possible 900 units) based on receiver operating characteristic statistics (ROC; AUC = 0.977). Studies are underway to evaluate more types of benign lesions in clinical Validation sets to determine if this marker panel will have clinical utility to distinguish other types of benign tissues from invasive breast cancers.
Future work: In collaboration with CEPHEID, a diagnostics company in California with instrumentation world-wide, we have transferred the QM-MSP technology to an automated system to detect multiple methylated target genes and a reference gene in 4 hours in tissue derived from core biopsy and FFPE sections. Independently, Cepheid developed STRAT4, an assay of ER/PR/HER and Ki67 expression status that can be determined within 2 hours. We have shown that both methylation and the STRAT4 assays can be performed from the same sample lysate. Together these two tests could provide fast, low cost and accurate diagnosis and subtyping of breast cancer.
TARGETING EARLY EVENTS IN BREAST CANCER
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Abstract Form

A wealth of evidence has demonstrated that the microenvironment in which a tumorigenic cell evolves is as critical to its evolution as the genetic mutations it accrues. However, there is still relatively little known about how signals from the microenvironment contribute to the early or late events in the progression to malignancy. To address this question, we have studied the stroma of human breast tissues and the signaling pathways that change in tissue highly predisposed to cancer.

We previously found that carcinoma-associated fibroblasts (CAFs), and the distinct ECM they deposit, can cause both premalignant and malignant mammary epithelial cells to assume a mesenchymal morphology which is associated with increased dissemination and metastasis, while benign reduction mammoplasty fibroblasts (RMFs) favor the maintenance of an epithelial morphology and constrain early dissemination, tumor growth, and metastasis. We found that a defining molecular characteristic of the above-described CAFs is their repression of CD36 and the reprogramming of the adjacent cells in the stromal microenvironment that ensues. CD36, a transmembrane receptor that coordinately modulates multiple pro-tumorigenic phenotypes including adipocyte differentiation, angiogenesis, cell-ECM interactions, and immune signaling, is greatly repressed in carcinoma-associated fibroblasts (CAFs) as well as in the multiple cell types present in tumor stromal desmoplasia. Using both in vitro and in vivo assays, we demonstrated that CD36 repression is necessary and sufficient to recapitulate the pro-tumorigenic phenotypes observed in breast tissues at high risk for cancer as well as tumor desmoplasia. Consistent with a functional role for this coordinated program in tumorigenesis, we observed that clinical outcomes were strongly associated with low CD36 expression. Given this information, we hypothesized that agents that have the ability to up regulate CD36 expression might have the potential to be a valuable tool in prevention and intervention of carcinogenesis. Recent data have indicated that stromal differences can be present in pre-malignant lesions.
DEFINING THE GENETIC BASIS OF IN SITU TUMOR PROGRESSION

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Advances in mammography have sparked an exponential rise in the detection of early-stage breast lesions, most commonly ductal carcinoma \textit{in situ} (DCIS). Although current treatments are curative for DCIS, epidemiological studies have established that over half of these lesions are indolent, and would never become life threatening even if left untreated. Consequently, many patients with DCIS are being systematically over-treated due to our inability to distinguish indolent lesions from those that will ultimately progress to invasive disease. Through analyses of patient breast tumors, we identified the transcriptional regulator SMARCE1 as a key driver of the invasive progression of early-stage and in situ tumors. We show that SMARCE drives invasion by regulating a transcriptional module of genes encoding for multiple secreted proteases that degrade basement membrane, the layer of extracellular matrix encapsulating all epithelial tissues. In functional studies, we show that upregulation of these proteases enables cancer cells growing \textit{in situ} within normal mammary tissues to invade into the surrounding stroma. In xenograft mouse models, we show that SMARCE1 is dispensable for primary tumor growth but is required for metastasis \textit{in vivo}. Mechanistically, SMARCE1 drives invasion by forming a SWI/SNF-independent complex with the transcription factor ILF3. In patients diagnosed with early-stage breast, lung and ovarian cancers, SMARCE1 expression is a strong predictor of eventual relapse and metastasis. Collectively, these findings establish SMARCE1 as an important driver of invasive progression in early-stage tumors.
PREVENTING BIDIRECTIONAL CROSSTALK BETWEEN BREAST CANCER CELLS AND RESIDENT ADIPOCYTES USING BIOACTIVE STRATEGIES

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Abstract Form

Adipocytes (fat cells) constitute ~90% of human breast and are the most prominent cell type in human breast. Adipocytes are not just energy-storing cells but are active endocrine cells that secrete cytokines, growth factors, and hormones to provide suitable microenvironment for breast cancer development. As breast cancer cells invade the basement membranes of the ducts/lobules and embark on invasive progression, there is increased molecular interaction between adipocytes and invading cancer cells. We hypothesized that this interaction further fuels the migration and invasion potential of breast cancer cells therefore inhibiting the crosstalk between cancer-associated adipocytes (CAAs) and breast cancer cells can potentially prevent/inhibit breast cancer growth and progression. Using various in vitro and in vivo model systems we show that adipocytes influence growth, invasion and migration potential of breast cancer cells. Using cytokine arrays, we discovered that cancer-associated adipocytes secrete increased levels of inflammatory cytokines and adipocytokines. We aim to develop novel, safe and effective strategies to abrogate adipocyte-breast cancer cell crosstalk. The importance of active constitutive agents in natural products has become increasingly apparent owing to their potential cancer preventive as well as therapeutic properties. Traditional Asian medicine has successfully used cones, bark and leaves from Magnolia plant species for their anti-thrombocytic, anti-inflammatory, anxiolytic, anti-depressant, antioxidant, antispasmodic, antibacterial and anticancer effects. Medicinal benefits of Magnolia species have been assigned to Honokiol (HNK), a natural phenolic compound isolated from an extract of seed cones from Magnolia grandiflora. Previous studies from our lab have shown that HNK inhibits breast carcinogenesis in vitro and in vivo. Our recent studies show the efficacy of HNK in inhibiting adipocyte-breast cancer cell crosstalk and elucidate the underlying molecular mechanisms. These studies lay the foundation of a stroma-centric approach to inhibit/prevent breast cancer progression focusing on endocrine-active resident adipocytes.
ARCHITECTURE IS THE MESSAGE FOR BOTH NORMAL BREAST AND BREAST CANCER: WHY LAMININS ROCK!
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Abstract Form

Research in the last three decades has been focused on the crucial role of extracellular matrix (ECM), and in particular laminin 111, as fundamental regulators in breast cancer. This molecule plays an extremely important role in organ- and tissue- specific mechanisms and function. The ECM of each tissue dictates how normal remains normal and how aberration in its components or the loss of laminins will eventually lead to genomic instability and cancer.

I will discuss also the important role of laminin and its receptors in drug resistance and therapy. Our current data connect laminin1 and laminin 5 to regulation of p53 and many other transcription factors in physiologically relevant 3D models.
Abstract Form

Introduction: Appropriate biomarkers measured in non-invasively collected nipple aspiration fluid (NAF) may be able to define risks and identify the early stages of breast tumor development. What target is selected for such a biomarker test will depend on the sensitivity of the analytical technique and on the potential for signal amplification.

Materials and methods: In a set of healthy and cancerous NAF samples collected at the University Medical Center Utrecht and the Erasmus Medical Center Rotterdam, The Netherlands, we have been investigating the biomarker potential of DNA promoter hypermethylation, microRNAs and, very recently, the whole transcriptome.

Results: Despite using a small amount of starting material (usually 10 microliters of NAF), biomarkers such as promoter hypermethylation, microRNAs and even the whole transcriptome can be studied and systematically analyzed in nipple fluid. Cancerous nipple fluid contains increased levels of promoter methylation and differentially expressed microRNAs that could potentially serve as biomarkers for early breast cancer detection. Whole transcriptome RNA sequencing will enable the characterization of all transcripts including both the coding (mRNA) and non-coding (microRNAs, long non-coding RNAs) RNAs, and therefore will be extremely valuable for understanding transcriptome dynamics and genetics-epigenetics interaction during carcinogenesis. As proof of principle, we recently successfully analyzed a NAF sample by RNA sequencing.

Conclusion: The successes and failures of techniques for early breast cancer detection in NAF may reveal promising directions for improvement in biomarker selection and assay development, eventually leading to a novel tool for early detection and a risk classifier to stratify high-risk women for prophylactic surgery or watchful waiting.
Here I will describe experimental studies of mammary tumorigenesis that were inspired and propelled forward by observations derived from computational and mathematical models. These in silico tools raised novel questions that might not have been investigated otherwise.

In the first study, computational network models were constructed to represent the features of cancer gene expression states from early hyperplasia to in situ ductal carcinoma to late state tumorigenesis and tissue invasion. With these large-scale network representations in hand, it was possible to identify stage-specific unique regulatory interactions and to select among these potential targets for therapeutic perturbation. Inferred gene regulatory network models were constructed using whole transcriptome expression profiling for progressive stages of mammary tumorigenesis in FVB C3(1)-SV40-TAg mice. These computational models predicted key mediator transcription factors critical for hyperplasia and ductal filling in the early stages of disease progression.

Candidate key mediator transcription factors were evaluated by RNAi targeting first in a mammary spheroid assay. Based on their ability to restore lumen formation and establish apical-basal polarization in mammary tumor spheroids, two candidate transcription factors, Hoxa1 and Hoxb13, were selected for further validation in vivo. siRNA targeting each of these transcription factors was formulated with lipidoid nanoparticles and delivered directly to hyperplastic mammary gland via intraductal injection. Silencing Hoxa1 resulted in a significant reduction in tumor incidence in FVB C3(1)-SV40-TAg. This represents a novel strategy to achieve localized silencing of a target gene in the mammary epithelium. Our results show that RNAi-based therapies can be similarly delivered intraductally in a mouse mammary cancer model, which both provides a more relevant animal model for breast cancer progression than fat pad injections and opens up the possibility for development of this class of non-cytotoxic siRNA-based agents as a localized treatment option.

In a second set of studies, the decision point at which lesions remain indolent or progress to locally invasive tumors was explored in the intraductal and fat pad environments. Titration of the tumor inoculum suggested that progression is described by bifurcation dynamics at a critical transition point. Intraductal seeding of tumors within the mammary duct enables the detailed study of how the tumor microenvironment and tissue context alters the frequency of critical point transitions.
Local treatments for ductal carcinoma in situ (DCIS) and breast cancer include local surgical excision. For some patients, polychemotherapy or tamoxifen or aromatase inhibitors are used with accompanying side effects and long term toxicity. Since the majority of DCIS and breast cancers originate in the epithelial cells lining the breast duct, we hypothesized that administration of agents directly into the breast ductal system (intraductal, i.duc) may result not only in reducing tumor volume and eradicating premalignant disease, but may also prevent the development of new lesions. The i.duc administration route allows for a more direct contact of the drug with mammary lesions (without dilution in the blood when injected intravenous), while limiting systemic toxicity. Our previous preclinical studies using both rat and mouse mammary tumor models (Cancer Res. 2006, Science Translational Medicine, 2011) have demonstrated that i.duc administration of Doxil, a pegylated liposomal preparation of doxorubicin, 5-fluorouracil (5-FU), and paclitaxel were very effective in treating and preventing mammary tumors in transgenic HER2/neu mice and carcinogen-treated rats, and achieved results superior to those treated by the intravenous route. However, Doxil and 5-FU injected i.duc induced mammary tumors in normal aged female FVB/N mice. Although effective, DNA damaging agents carry the risk of iatrogenic tumors in the long term (Breast Cancer Research and Treatment, 2012). We hypothesized that i.duc administration of the selective estrogen receptor degrader (SERD), fulvestrant, will be a safe and effective route for treatment of DCIS and for long term prevention of cancer by eliminating occult precancerous cells in the diseased ductal tree. Mice (NSG) received an injection of MCF-7-luc cells in the fourth mammary gland on both sides of the through the teat. Following the injection of the substrate, luciferin, the growth of the tumors inside the duct could be observed noninvasively at regular intervals by IVIS imaging. The mice developed DCIS lesions by day 10 that progressed to invasive cancer by day 30 and metastasize to the lung by day 60. Mice were divided into three groups- vehicle, intramuscular (i.m) fulvestrant and i.duc fulvestrant at human equivalent doses. Mice were sacrificed 75 days later. By IVIS imaging, mammary lesions did not increase in size significantly over time in mice that received fulvestrant i.duc, while the tumors in mice treated i.m with the same dose of the drug continued to grow for 46 days and then showed regression. Tumors treated with fulvestrant i.m started regressing at a time that coincided with the progression of DCIS to invasive cancer. Regression achieved by i.duc injection of fulvestrant was more significant compared to im injection of the drug and vehicle control. After sacrifice, mammary
glands were examined as whole mounts by carmine staining and histopathology performed following Hematoxylin and Eosin (H &E) staining of the formalin fixed mammary tissue. Lung, liver, and kidney were examined grossly following staining with picric acid for distant metastasis, and for microscopic metastasis following H&E staining. Comparison of markers of proliferative activity (Ki67), Estrogen receptor, and CD31 is ongoing. Similar results were obtained with N-methyl N-nitrosourea-induced mammary tumors in Sprague Dawley rats.
A RANDOMISED CONTROLLED TRIAL OF DUCT ENDOSCOPY IN PATHOLOGICAL NIPPLE DISCHARGE
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Abstract Form

Introduction: Breast cancer associated with nipple discharge ranges from 5-15%. A higher risk is associated with a palpable mass, radiological abnormality, age or if the discharge is blood stained. In this study, we evaluated patients with single duct persistent nipple discharge in the absence of any clinical mass and with no radiologically suspicious lesion. Conventional surgery to nipple ducts is undirected and targeting any causative lesion by duct endoscopy prior to standard surgery may enable smaller resection volumes, potentially reducing surgical complications.

Patients and Methods: Patients were randomised to receive either diagnostic duct endoscopy or not, immediately before the planned surgical procedure under a general anaesthetic. Any causative lesion identified was localized with an expanding rhomboid wire passed down the working channel (Polydiagnost, Germany). Patients randomised to standard surgery proceeded directly to microdochectomy and major duct excision without duct endoscopy. The localization procedure allowed the discharging duct system to be excised beyond the identified lesion. The study aimed to randomise 70 procedures on the basis of successful visualisation with 80% power and one-sided 5% alpha. The endpoints were: (a) successful visualisation of a pathological lesion in patients randomised to have duct endoscopy; (b) compare the pathological yield between duct endoscopy (DE) and no duct endoscopy (NDE) groups; (c) compare the specimen resection size between both groups.

Results: The final analyses included 68 breasts in 60 patients, 32 women in the DE group and 36 in the NDE group. The median age was 49 (range 19-81 years). The follow-up in the DE group was 5.4 (IQR 3.3-8.9) years and the follow-up in the NDE group was 5.7 (IQR 3.1-9.0) years. The test characteristics of duct endoscopy in identifying any lesion was sensitivity 80% (95% CI 51.9-95.7), specificity 70.6% (44.0-89.7), PPV 70.6% (44.0-89.7) and NPV 80% (51.9-95.7). The commonest benign lesion identified was a papilloma with sensitivity 83.3% (51.6-97.9), specificity 94.7% (74.0-99.9), PPV 90.9% (58.7-99.8) and NPV 90.0% (68.3-98.8). The sensitivity of duct endoscopy in identifying duct ectasia was 42.1% (20.3-66.5), specificity 100% (73.5-100), PPV 100% (63.1-100) and NPV 52.2% (30.6-73.2). Diagnostic duct endoscopy was not more likely to result in histologic yield to account for the nipple discharge: any lesion p=0.143, papilloma p=0.305, duct ectasia p=0.628. Five patients were identified with DCIS, 3 in
the DE and 2 in the NDE group). The number of DCIS events was too small to evaluate significant test characteristic values. The median volume (IQR) of the surgical resection specimen in the DE group was 4875 (2016-8280) mm$^3$ versus median 4455 (2400-7000) mm$^3$ in the NDE group, $p=0.577$.

**Conclusions:** Diagnostic duct endoscopy is a useful tool in the identification and localisation of causative abnormalities. Benign papillomas are the commonest identifiable source of nipple discharge. Duct ectasia may be under-reported by duct endoscopy. The pathological yield of a benign or malignant diagnosis, surgical resection size and complication rates were not influenced by pre-operative duct endoscopy.
FIRST REAL-TIME MICROSCOPIC IMAGING OF BREAST DUCTS BY DUCTOSCOPY INTEGRATED OPTICAL COHERENCE TOMOGRAPHY: A FEASIBILITY STUDY

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Abstract Form

This study aimed to determine whether the imaging of intraductal epithelium or microcalcifications is feasible by the use of optical coherence tomography (OCT) via intraductal route. Eighteen (n=18) mastectomy specimens were enrolled in the study. Fourteen (n=14) specimens were able to cannulated by ductoscopic instruments and distended by saline during ductoscopy. Four (n=4) out of eighteen specimens were with nipple discharge clinically. The insufficiency during cannulation was due to specimens including narrow ducts without nipple discharge. Twenty-eight (n=28) isolated ducts were explored with ductoscopy to ensure whether the ductoscopic trocars were inside the ducts. The catheter of OCT was inserted through the ductoscopic trocars and examined whether it is showing microscopic layers of ductal lining. We showed microscopically the ductal epithelium, basal membrane, bifurcations, microcalcifications, and unspecified lesions with high-resolution OCT. The impact of the study was achieving microscopic images intraductally without dissected histopathologic tissues. This is the first study which confirms the feasibility of a new real-time imaging method, intraductal OCT, that were able to show microscopic structure of breast epithelium and microcalcifications via intraductal access.

Key words: Optical Coherence Tomography, OCT, Ductoscopy, Breast Cancer, Nipple Discharge

Table 1. OCT imaging of a breast duct with a calcified lesion