Using Next Generation Science to Understand the Normal Breast and the Development of Breast Cancer

Conference Report

Dr. Susan Love Research Foundation is committed to performing and advancing research that will lead to the discovery of what causes cancer to develop in the human breast. As part of this effort, the Foundation hosted the 8th International Symposium on the Breast in Santa Monica, Calif., Feb. 19-21, 2015. More than 120 forward-thinking clinical researchers, epidemiologists, pathologists, basic scientists, translational investigators, and breast cancer advocates from six countries attended this year's conference, "Using Next Generation Science to Understand the Normal Breast and the Development of Cancer."

The Symposium was organized around three central topics: the anatomy and molecular biology of the breast and cancer risk; the microenvironment and microbiome of the normal and cancerous breast; and clinical applications of next generation science. During the conference, attendees also had the opportunity to observe live demonstrations of nipple aspirate fluid collection, ductal lavage, and ductoscopy. A Public Panel allowed the community to hear highlights of the Symposium and gain insight into new directions in breast cancer research.

On the final day of the conference, Dr. Susan Love Research Foundation awarded a total of $70,000 to support four multidisciplinary consortia formed at the Symposium that will conduct research focused on using next generation technology to investigate the human breast and how it develops cancer.

Introduction

Recent advances in technology have created new opportunities in breast cancer research. Dr. Susan Love Research Foundation's 8th International Symposium on the Breast fostered an intimate think-tank environment where researchers, clinicians, and breast cancer advocates

could discuss and explore ways to use these new technologies to obtain long-sought answers to critical questions on how breast cancer develops. Each researcher who spoke at the conference brought a unique or novel perspective to the overarching questions driving the Symposium: How does breast cancer develop? What do we need to do and learn so that we can prevent breast cancer from happening in the first place?

The Anatomy and Molecular Biology of the Breast and Cancer Risk

Barry Gusterson, PhD, professor of pathology at the University of Glasgow, Scotland, opened the conference with his presentation, "The Unknown Knowns in Breast Cancer and Breast Biology—and Then There is DCIS—A Pathologist's Perspective."

Dr. Gusterson focused attention on methods used to classify breast tumors that researchers often think of as absolutes, but are not. Many assumptions about the human breast are based on findings from mice studies, he said, "but what is seen in mice is not necessarily transferrable to humans." For example, the terminal end buds studied in mice are rarely seen in the human developing breast. Dr. Gusterson believes all ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS) start in the terminal duct lobular unit, and that the differentiation is a misclassification. He suggested that definitions of BRCA1 and medullary tumors have become intertwined, noting that only 13% of women with medullary tumors have a family history of breast cancer. And he highlighted a need to improve the assays that test for hormone and HER2 receptor status to ensure accuracy and quality control. Lastly, Dr. Gusterson asked attendees to consider whether the new technologies being used to identify subtypes of breast cancer and the focus on new targets are advancing the field or leading researchers to lose sight of the importance of the estrogen receptor. "Estrogen is where it is and it is where it should stay," he said. There are "fashions in research, but we must not throw away what we know."

Ellen Carpenter, PhD, professor of psychiatry and biobehavioral sciences at UCLA School of Medicine, studies reelin, an extracellular matrix glycoprotein that helps regulate the lamination and organization of migratory brain cells. Mammary breast epithelial cells are also highly migratory, which led Dr. Carpenter to investigate the role of reelin signaling in breast

development. These studies show that disrupting reelin signaling significantly alters the development of the mouse mammary gland and produces highly abnormal ductal trees, likely through local stromal-epithelial interactions. They also show that mouse mammary tumor cells do not express reelin or Disabled-1(Dab1), a regulator of reelin signaling, but do express reelin receptors. In human cell lines, Dr. Carpenter's team found that reelin does not inhibit the migration of mammary tumor cells. She is now exploring the role that reelin signaling has on breast cancer cells inside the duct as well as on the migration of cells in invasive and metastatic breast cancer.

Thea D. Tlsty, PhD, professor of pathology and director of the Center for Translational Research in the Molecular Genetics of Cancer at the University of California, San Francisco, conducts research on breast stem cells. Normally, cells arrest when they are damaged. Dr. Tlsty identified rare cells within human adult breast tissue that can acquire a phenotypic plastic state that is sensitive to environmental programming, that continue to grow when damaged, and that differ from other pluripotent cells previously described in the literature. Dr. Tlsty's team made beating heart cells, pancreatic tissue, and bone and bowel cells from these human endogenous Plastic Somatic cells (ePS cells), and they demonstrated that all three types of human tissue—ectodermal, endodermal, and mesodermal—can be created from these cells in a teratoma assay. This is "the holy trinity of pluripotency," said Dr. Tlsty, "because it is what is found in embryonic stem cells." It also supports the hypothesis that breast tumors may be created from a single pluripotent cell.

The next speaker, Pepper Schedin, PhD, professor of cell and developmental biology in the School of Medicine at Oregon Health & Science University, discussed postpartum breast cancer. Each year in the U.S., between 12,000 and 15,000 cases of postpartum breast cancer are diagnosed, and women diagnosed with postpartum breast cancer have a three-fold increase in risk for metastases and death. "This poor prognosis is a feature of postpartum diagnosis—not diagnosis during pregnancy—and it is independent of the biological subtype of the tumor," said Dr. Schedin. In rat models, cellular mechanisms of postpartum lobular involution and remodeling suggest that involution promotes tumor growth. A study of adjacent normal tissue from 151 women enrolled in The Young Women's Breast Cancer Translational Program, Dr. Schedin found that epithelial content and lobule subtype return

to pre-pregnancy state by 18 months postpartum, and that postpartum involution shares numerous attributes with the tumor microenvironment, including wound-like immune cell influx. This suggests that the microenvironment of postpartum involution promotes breast cancer metastasis, and because this involution occurs in a narrow window of time, it creates the opportunity for interventional treatment. Dr. Schedin reported that in rodent models a two-week treatment with COX-2 inhibition by NSAIDS blocked the tumor promotion of involution. Noting that postpartum breast cancer patients are easily identifiable, Dr. Schedin called for more research on ways to target and reverse the changes in the breast microenvironment that increase postpartum breast cancer risk.

RNA-Sequencing data is used to explore the developmental biology and genetics of the normal breast. Susan E. Clare, MD, PhD, a research associate professor in breast surgery at the Feinberg School of Medicine at Northwestern University, and colleagues produced RNA-sequencing data from breast tissue donated by 20 healthy premenopausal women to the Susan G. Komen Tissue Bank at the Indiana University Simon Cancer Center. This research identified 255 genes, representing 1.4% of all genes, that had statistically significant differential expression between the two phases of the menstrual cycle, with the overwhelming majority (221; 87%) having higher expression during the luteal phase. The team also downloaded RNA-Sequencing data from The Cancer Genome Atlas data portal on histologically normal adjacent breast tissue. In this "normal" tissue, translation and cellular protein metabolism were two of the top biological processes affected by the BRCA mutations, underscoring, as Dr. Clare said, "the adjacent normal tissue is not normal." The adjacent normal tissue also contained APOBEC3C—proteins that may be RNA editing enzymes and affect cell cycle control, which suggests that the cancer may be going into the adjacent normal tissue and turning on APOBEC3C before there is histological evidence in the tissue that the cancer has spread.

Continuing the interrogation of "normal" tissue, Amy C. Degnim, MD, professor of surgery at the Mayo Clinic in Rochester, Minnesota, presented research on normal breast tissue, reduction mammoplasty (often used in research as a benign control), and benign breast disease. In 2012, Dr. Degnim published studies that found fibrocystic change is common in "normal" tissue and that reduction mammoplasty is more similar to benign breast disease

tissue than normal tissue. Additional studies exploring histologic features and the immune microenvironment in these tissues found that normal breast tissue has histologic features more consistent with lower breast cancer risk—such as less epithelial proliferation and more complete involution—than benign breast disease tissue. A quantitative characterization of immune cells in individual breast lobules in normal tissue found the cells were present in consistent locations in normal breast tissue and were predominantly in the lobules. These immune cells included both myeloid and lymphoid components that were present even when immune infiltrates were not apparent on H&E stain and, within the lobules, CD8+ cells and dendritic cells were directly integrated with the breast epithelium. Dr. Degnim concluded that these findings suggest that normal breast tissue contains an active and dynamic mucosal immune system and that immune modulation might be a mechanism for cancer prevention.

Advancing the conversation, Xuezheng Sun, PhD, from the department of epidemiology at the University of North Carolina at Chapel Hill, discussed the molecular epidemiology of normal breast tissue. The breast is a dynamically changing organ and there may be more times when it is more susceptible to carcinogenesis. Mammary gland involution in adulthood may be one of these time periods and it is possible that environmental exposures that occur during this time period cause molecular or histological changes that can increase risk. Dr. Sun reported that gene expression analysis, histological imaging techniques, and radiological imaging techniques of normal breast tissue from women who had mammoplasty found that obesity, parity, and breastfeeding cause changes in the histology of the normal mammary gland that affect molecular signaling and gene expression—and that coincide with changes in mammographic density. This suggests the relationship between mammographic density and risk is affected by breast histology and the breast microenvironment.

The discussion of risk factors turned in a different direction with Mina Bissell, PhD, a distinguished scientist at the Lawrence Berkeley National Laboratory, asking, "Why don't we get more cancer?" Dr. Bissell explained that it is not only the genetic mutations that develop inside the cell but the area in which that cell resides—the extracellular matrix—that must be primed for the cell to change from normal to cancerous. She also described how her laboratory's use of 3-dimensional models of the normal mammary gland and mammary

tumors from both mice and humans has given them insights into the complex dynamic relationship between the cell and the extracellular matrix. "Cancer growth and malignant behavior are regulated at the level of tissue organization," she explained, "and that architecture is dependent on the microenvironment and the extracellular matrix—which can create genomic instability." Currently, Dr. Bissell is studying laminin, a protein found in the basement membrane that influences, among other things, cell migration and survival. She reported that laminin regulates both the activation of p53, a tumor suppressor gene, and nitric oxide, which has provided new insights into cancer development. "What is important in cancer is not just the mutations. You can sequence many cancers, but you need to pay attention to the architecture," Dr. Bissell said. "As we age, that architecture falls apart. We can learn to keep cancer dormant, but we can't eradicate it because we can't eradicate aging."

The next presentation shifted the focus from the breast tissue to the breast ducts. The ducts are lined by a single layer of columnar epithelium that forms a selectively-permeable barrier between the external and internal environments. These ductal barriers are regulated by a series of multi-protein intercellular adhesion complexes including tight junctions. Ann Hopkins, PhD, a lecturer at the Royal College of Surgeons in Ireland, studies the tight junction protein Junctional Adhesion Molecule-A (JAM-A), a transmembrane molecule that provides polarity that is necessary in the normal breast. JAM-A overexpression correlates with a poor patient prognosis—large tumors, high-grade tumors, increased metastasis and HER2 expression. Dr. Hopkins identified a strong correlation and cross regulation between JAM-A and HER2 as well as indicators that suggested JAM-A was controlling HER2. Based on these findings, Dr. Hopkins and her team have begun studying a JAM-A antagonist called KB3.

Ann Ramsdell, PhD, an associate professor of cell biology and anatomy at the University of South Carolina School of Medicine, discussed research on the laterality of breast cancer. Breast tumor incidence and metastatic involvement differ according to whether the primary tumor develops in the left versus right breast. (About 55% of breast tumors are diagnosed in the left breast, and about 45% are in the right breast.) Dr. Ramsdell's research on normal and neoplastic mouse models found asymmetrical differences in gene expression and cellular content, which included a population of multi-potent, ductal epithelial cells with self-renewal

properties, termed mammary stem cells (MaSCs). This differential expression also held true for EGFR expression, and tumor response to lapatinib, a kinase inhibitor used to treat HER2+ breast cancer. These findings suggest that whether the tumor is in the right or left breast could affect drug response and survival.

Laterality is only one aspect of differentiation. There is also a growing awareness that there is heterogeneity within a single tumor. William Coleman, PhD, a professor in the department of pathology and laboratory medicine at the University of North Carolina, discussed the process of field cancerization, and how it may help explain a this heterogeneity. Cancer was typically thought of a growth that occurs in a linear progression. Field cancerization is an approach that suggests the initial alterations create a field that leads to additional alterations that gives rise to a lesion on the altered field. This suggests that multiple lesions can develop in the same field, giving rise to individual tumors that may be similar or distinctly different. It also suggests that adjacent normal tissue is not normal at all but is actually neoplastic. This is supported by molecular studies that have found that breast epithelial cells that surround a tumor can carry cancer promoting mutations, epimutations, or other molecular alterations. By taking account the impact of the field on the tumor as well as tumor heterogeneity, Dr. Coleman said, "field cancerization may not only explain tumor development but have implications for how we think about cancer treatment."

Returning to the ducts, Andrew Maidment, PhD, an associate professor of radiology at the University of Pennsylvania in Philadelphia, discussed ongoing research into topological features of the breast duct. The breast ducts contribute to underlying patterns of mammograms, and radiologists use these normal patterns to look for what is discordant and may be a lesion. Dr. Maidment uses galactography to study these patterns with the aim of determining which aspects of the ductal branching patterns are correlated with breast development and cancer risk. These studies, which use computer modeling to compute branching probabilities, have found a significant correlation between ductal topology and seven parenchymal texture descriptors. This early research suggests that ductal topology can directly influence the parenchymal patterns seen in various women.

Next, Clare Isacke, PhD, from the Institute of Cancer Research in London, discussed anatomy of fluid yielding ducts in the breast and their relationship to breast cancer. Ductal lavage is a technique that allows researchers to study ductal fluid. Dr. Isacke investigated the accessibility and patency of the breast duct in 58 women scheduled to have a mastectomy. They obtained ductal fluid from 47 of these women and, of these, 37 were successfully cannulated. After infusing the ducts in the breast tissue that had been removed with colored resins, the study showed that the ducts that produced fluid and that were cannulated were concordant with the cancer-affected segment of the breast, allowing Dr. Isacke to conclude that ductal access to the cancer-containing segment of the breast is feasible in the majority of patients.

Ductal (nipple) fluid analysis also holds the potential for early breast cancer screening. Paul J. Van Diest, MD, PhD, head of the department of pathology at University Medical Center Utrecht, Netherlands, discussed a study that analyzed DNA methylation levels by QM-MSP in nipple fluid samples obtained from 49 healthy women and from the healthy or cancerous breast of breast cancer patients. Despite low levels of methylation in the fluid, the study identified common tumor suppressor genes that could potentially be used as a biomarker for early detection. Dr. Diest also reported that similarities seen in fluid taken from a woman's cancerous and normal breast "suggest something is happening in the healthy breast," adding to the evidence that the "healthy" breast may not be "normal."

Spontaneous nipple discharge can be the result of a papilloma, duct ectasia, hyperplasia or malignancy (DCIS). Mammary ductoscopy allows surgeons to see inside the duct and help identify the cause for the discharge. It has also been used to guide surgical margins during lumpectomy surgery; for early diagnosis and screening of high risk patients; and in studies exploring intraductal therapies. Sheldon Feldman, MD, FACS, professor of clinical surgery at Columbia University Medical Center in New York City, a leader in this field, began performing ductoscopy in 2003. He explained how the procedure is performed, from duct cannulation and dilatation of the ductal orifice to insertion of the introducer and the ductoscope, and showed videos of the ductoscopy procedure. He also discussed ongoing research into autofluorescence and optical biopsies along with the more recent introduction

of cytology brushes, microbaskets, and biopsy tools that can be used during the procedure. The following day, Dr. Sheldon demonstrated the ductoscopy procedure on a live volunteer.

The University Medical Center in Utrecht began offering ductoscopy as a treatment option for women with pathologic nipple discharge in 2010. Arjen J. Witkamp, MD, discussed the University's use of interventional ductoscopy. To date, approximately 110 women (and 1 male patient) have undergone ductoscopy, with basket extraction performed when intraductal lesions were found. Ductoscope introduction was successful in 71 (87%) of these patients, with abnormalities visualized in 53 (65%); surgery was avoided in 68%. In 27 of the 34 attempted ductoscopic extractions, the lesion could be removed, and a malignancy was diagnosed in 5 (6.1%) patients. However, diagnostic accuracy was low: No abnormalities were found in 22% of the women and 47% had persistent pathologic nipple discharge after the procedure. Dr. Witkamp and his colleagues developed a technique in which they insert a laser fibre into the ductoscope cannula for laser ablation, which may ultimately improve efficacy. They are also studying autoflorescence for enhanced diagnostic sensitivity during ductoscopy, with a phase II study in high-risk patients or patients with breast cancer undergoing mastectomy currently ongoing.

In closing, attendees were provided information about and encouraged to join consortia and compete for pilot grants to apply next generation science to the study of the human breast. Pilot grants would be awarded on the final day of the Symposium.

The Microenvironment and Microbiome

The second day of the Symposium opened with Don E. Ingber, MD, PhD, at the Wyss Institute at Harvard University in Boston, discussing the emerging field of biologically inspired engineering and his team's ability to build 3-dimensional models of living human organs, called "Organs on Chips," that can be used in place of animal models to study diseases like cancer and test promising drug treatments. Dr. Ingber described how variability in gene expression and the extracellular matrix interact to govern cell phenotype switches that can both form—and reverse—tumor development. He also discussed the potential to use the differentiation cancer therapies used in blood cancers to induce cells in breast and

other solid tumors to revert from cancerous to normal by changing the tumor microenvironment. This process could potentially be studied on a chip of a human breast cancer.

The intraductal approach to treatment creates the potential to target ductal carcinoma in situ (DCIS) with an anti-cancer drug while lowering systemic side effects. Patrick J. Sinko, PhD, RPh, professor at Rutgers, The State University of New Jersey, described his studies on a doxorubicin poly(ethylene glycol) (PEG) nanocarrier that could be used for this purpose. The approach attaches doxorubicin, which is widely used to treat breast cancer, to PEG carriers that are able to keep the chemotherapy drug inside the duct and out of the bloodstream. Animal studies conducted by Dr. Sinko's team evaluating different molecular weights and structures of the nanocarriers suggest the approach may be effective in humans.

Sara Sukumar, PhD, professor at Johns Hopkins University School of Medicine in Baltimore, also discussed treatment and presented her research on intraductal radioimmunotherapy for DCIS. Dr. Sukumar's experimental safety studies on the use of chemotherapy for intraductal therapy found that the drugs could cause tumors to develop in normal mice. Radioimmunotherapy provides targeted delivery of radiation. The radiation—alpha particle emitters—give out highly focused energy along a short path length; the delivery vehicle is the HER2-targeted therapy trastuzumab (Herceptin). Animal studies showed the therapy was effective when given intraductally in DCIS-like xenograft mouse models. Dr. Sukumar reported that at the time of the Symposium, the mice had been followed for 8 months and no tumors had formed. Additional studies performed by Dr. Sukumar's team showed that the treatment can also reach metastatic and early stage tumors.

Women who carry a BRCA1 mutation are at increased risk of developing breast cancer. The most effective treatment to reduce breast cancer risk is a bilateral mastectomy and salpingo-oophorectomy. Jolien DeGroot, PhD, from the University Medical Center, Utrecht, is investigating whether intraductal treatment can offer an alternative to prophylactic surgery for high-risk women. She presented a study on whether a combination of intraductal cisplatin and a PARP inhibitor could slow or stop growth of BRCA1-associated mammary

tumors in a mouse model. The study found that the treatment delayed tumor onset for more than two years, but did not prevent it.

Herbert Kim Lyerly, MD, a professor of surgery at Duke University in Durham, North Carolina, began his presentation by asking, "How large is a cancer cell—and how many of these cells must be present in the breast before they can be detected?" The answer, he explained, is that about 1000 cancer cells are the size of a period, and about one billion cancer cells form a lump of 1cm. Currently, the smallest tumor that can be detected is about 4mm. Dr. Lyerly is developing molecular probes that might be able to detect aberrations of biochemistry and signaling that occur before anatomic changes that can be seen develop in the breast. As proof of concept, his team studied heat shock protein 90 (HSP90), which is present in high concentration in breast cancer cells of all molecular subtypes. Although HSP90 is in every cell, an HSP90 inhibitor will accumulate in tumor cells. Dr. Lyerly reported that by altering an existing HSP90 inhibitor and linking the molecule to a short chemical bridge, his team was able to detect small collections of intraductal tumor cells by near infrared imaging in mouse models. They hope to pursue this work in a phase I study.

Keeping the focus on early detection, Cathy Moelans, PhD, from University Medical Center Utrecht, discussed research into the use of microRNA expression profiling in nipple aspirate fluid (NAF) as a diagnostic biomarker. She presented data from a study that looked at NAF samples from 18 healthy women and 10 women with breast cancer. On average, 220 microRNAs were found in each sample. Fifteen of the 20 most highly expressed were immune related, which is similar to what is seen in microRNA analyses of breast milk. Two of these are implicated in carcinogenesis and four have been reported as potential circulating diagnostic markers for breast cancer. This pattern of dysregulation fits perfectly in the constitutive activation of the IL6-STAT3 pathway, Dr. Moleans said, suggesting a potential link between inflammation and carcinogenesis.

Clare Isacke, PhD, speaking on behalf of her colleague Gerald Gui at the Institute of Cancer Research in London, discussed DNA promotor hypermethylation in breast duct fluid. Methylation is seen across the genome, but the patterns shift with cancer. The study compared methylation of cells found in ductal lavage fluid to cells seen in tumor tissue,

adjacent "normal" tissue, contralateral ductal lavage fluid, and ductal lavage from healthy women. She reported that patterns of DNA methylation were similar in the ducal lavage samples and paired cancer tissue and that the comparisons of cancer tissue to "normal" adjacent tissue were sensitive, but not highly specific, suggesting a field effect. Although more methylation was found in the cancerous breast than in the matched contralateral, methylation was high in both. Lastly, there was no correlation in methylation from the cancerous breast and from healthy women, suggesting a potential for differentiation.

The ability to detect circulating tumor DNA in metastatic breast cancer could help doctors determine when to start or change cancer treatments. Mary Jo Fackler, PhD, at the Sidney Kimmel Cancer Center at Johns Hopkins, described a new quantitative multiplexed methylation-specific PCR assay to detect circulating tumor DNA. The 10-gene assay includes both novel and known breast cancer hypermethylated markers. In training and test patient sets, the cMethDNA assay accurately detected metastatic breast cancer with a sensitivity of 91% and specificity of 100%, compared to individuals without cancer. A pilot study found that the test reflected patient response to chemotherapy, and an analysis of a larger prospective trial is now underway. Dr. Fowler reported that core methylation signature present in the primary breast cancer was found in metastatic tissue and blood collected at autopsy. This suggests, she said, "that breast cancer markers identified at diagnosis persist and that the signature could be used over time to follow patients and test them at regular intervals to detect early recurrence and redirect treatment if therapies are not working."

Several epidemiologic studies have identified a low but significant association between breast cancer risk and levels of blood estrogens and androgens. Robert T. Chatterton, PhD, professor of obstetrics and gynecology at the Feinberg School of Medicine at Northwestern University in Chicago, presented research findings that compared steroid concentrations in nipple aspirate fluid (NAF) obtained from the contralateral breast of women with breast cancer and women without breast cancer. The study included 153 premenopausal and 155 postmenopausal women. There was no association between estradiol and the presence of a breast tumor, but NAF from the contralateral breast did have had a significantly higher level of DHEA and a significantly lower level of progesterone than NAF from healthy women. The study also found that the tumor's hormone status was associated with different NAF

hormonal levels and that, except for progesterone, the associations of steroid concentrations with breast cancer were limited to breast fluid and not seen in serum. Dr. Chatterton said more research is needed to determine if hormone levels in the contralateral breast exist prior to the initial tumor and represent a precondition for the development of the tumor or if the tumor is affecting the contralateral breast.

This research was supported in part by Dr. Susan Love Research Foundation.

Norman Javitt, professor of medicine at New York University Medical Center, discussed 27-hydroxycholesterol, an estrogen-mimetic oxysterol that has been found in breast tumor tissue as well as in the contralateral breast of women with ER-positive breast cancer. The drug tamoxifen blocks tumor growth by binding to the estrogen receptor. Tamoxifen can also lower plasma cholesterol levels, possibly through a process that increases several sterol intermediates that can be further metabolized to 27-hydroxycholesterol and other oxysterols. Dr. Javitt suggested that this phenomenon may explain why the ATAC trial found that combining tamoxifen and an aromatase inhibitor was less effective than tamoxifen or an aromatase inhibitor alone. He also noted that tamoxifen's effect on local accumulation of 27-hydroxycholesterol may be genetically determined, which could help explain why some women's tumors stop responding to tamoxifen.

The second day of the Symposium ended with a live demonstration of NAF/ductal lavage by Susan Love, MD, and a live demonstration of ductoscopy by Sheldon Feldman, MD.

Next Generation Clinical Applications

The third and final day of the Symposium opened with a presentation by James Hicks, PhD, research professor at Cold Spring Harbor Laboratory in New York, on the use of single cell genomics to track the initiation, earliest stages, and evolution of breast cancer. His research team has applied single cell DNA copy number profiling obtained by NextGen Sequencing to study the heterogeneity of breast tumors and metastases as well as the genomic analysis of rare circulating tumor cells in early and late stage breast cancer patients. Dr. Hicks described the methods they use for single cell copy number variation (CNV) analysis to identify and track the lineage of cancer cells in patients and animal models, how they obtain the cells from biopsies and blood samples, and how it "provides an inexpensive, mathematically

accessible means to assess genomic progression" and generate an evolutionary history of the tumor. He discussed clinical trials using the approach, including a neoadjuvant breast cancer study exploring whether CNV can identify which types of cells respond to treatment.

Breast cancer risk increases as a woman ages, but the reasons for this increased susceptibility are not well understood. Mark LaBarge, PhD, staff scientist at Lawrence Berkeley National Laboratory, is looking for answers by studying the human gene expression changes that occur in the mammary epithelia as women age. If these epigenetic changes accumulate with age, it would suggest that aging could degrade the microenvironment, allowing cells to get out of the duct and grow. But it is also possible that epigenetic changes alter the function of the cell, which then changes the tissue. To explore these possibilities, Dr. LaBarge studies how breast tissue responds to various simulated controlled microenvironments. This research has found that mammary progenitors accumulate with age because they lose sensitivity to microenvironmental directives and that aging fundamentally changes mechanical triggers and cell differentiation programs. In conclusion, he said, "Aging is like a bad game of telephone. The messages during aging trickle through the stroma in the breast to the basement membrane and myoepithelial cells that then change the inner layer, where the cancer comes from. If we can manipulate and control this game of telephone, we could decrease the breast cancer susceptibility that increases with aging."

Camilla Urbaniak, PhD, of the Lawson Health Research Institute at the University of Western Ontario, discussed microbes in the breast. The body contains more bacteria than cells, and areas of the body, like mammary tissue, that were once thought to be sterile actually contain microbes. Dr. Urbaniak's research identified a diverse community of bacteria in breast tissue and found that bacterial communities differ between healthy women and women with breast cancer. She questioned whether certain drug treatments that change the microbiota, such as antibiotics or chemotherapy, might promote breast cancer development and suggested that antibiotic or probiotic manipulation of breast microbiota might have a role in breast cancer prevention or treatment.

Delphine Lee, MD, PhD, of the John Wayne Cancer Institute at Providence St. John's Health Center in Santa Monica, California, presented research on the relationship between

the immune system and the microbiome of breast tissue. Her team found that bacterial load in breast cancer tissue is inversely correlated to the progression of disease. They also found that antimicrobial gene expression is comparatively higher in breast tissue from healthy subjects. Overall, she reported, microbiome signatures in breast cancer patients are similar when comparing healthy adjacent tissue to the tumor tissue. However, not all the bacteria are equally abundant in both breasts. Dr. Lee said their preliminary data suggest that bacteria found in healthy adjacent tissue stimulate differential cytokine responses that may shape the local tumor microenvironment and contribute to immune surveillance.

With the next speaker, James Holland, MD, professor of medicine at Mount Sinai Hospital in New York, moved the conversation from bacteria to viruses. The mouse mammary tumor virus (MMTV) causes cancer in mice. Dr. Holland's team identified and studies a nearly identical virus founding in human, HMTV. Dr. Holland reported that HMTV is found in about 40% of women with breast cancer. It is not found in normal breast tissue, which suggests HMTV is an infection, and not inherited. In a study of 32 women with metastatic breast cancer, 30 had the virus in chest or abdominal fluid. Dr. Holland reported findings from lab experiments that showed HMTV in breast cancer cells can infect normal mammary cells and normal blood cells, and that these infected cells acquire some molecular characteristics typical of cancer cells. In conclusion, Dr. Holland said, these findings, taken as a whole, suggest HMTV is credibly a causative factor for human breast cancer.

Faith Balci, MD, assistant professor of surgery, Acibadem University Atakent Hospital, Istanbul, Turkey, in affiliation with Dr. Feldman at NY-Presbyterian Hospital, Columbia University, presented preliminary results from research on Human Papillomavirus (HPV) and breast cancer. Previous studies have looked for HPV DNA in breast cancer samples. Dr. Balci is investigating whether HPV is found in precancerous lesions, such as breast papilloma or atypical ductal hyperplasia, as well as breast tumors. To date, 27 intraductal papilloma extracted through ductoscopic papilloma extraction and 18 breast tumors have been analyzed using real time PCR with broad-spectrum genotyping. Dr. Balci reported a high prevalence of HPV was found in both groups, with HPV-11 the most common. In addition, papilloma and tumors that tested positive for HPV, stained weak or negative for

hormone and HER2 receptors. Dr. Balci concluded this suggests HPV may have triggered the development of these papilloma and cancers.
This research was supported by a pilot grant from Dr. Susan Love Research Foundation.

Atilla Soran, MD, MPH, FACS, professor of surgery at Magee-Women's Hospital of University of Pittsburgh Medical Center, presented findings from a biomarker feasibility study investigating whether MUC1 and cyclin B1, which have been found in breast tumors, are present in high concentrations in nipple aspirate fluid (NAF) and if there is a correlation between NAF antibodies and tumor characteristics. The study analyzed 123 NAF samples obtained from 76 women, collected before surgery from the precancerous/cancerous breast and/or the contralateral breast. Cyclin B1 antibodies reached statistically significant difference to discriminate cancer or a high-risk lesion from normal breast tissue. In addition, high level of NAF anti-cyclin B1 IgG was significantly associated with more aggressive tumor characteristics. Based on these findings, Dr. Soran intends to conduct larger studies on NAF and serum anti-cyclin B1 IgG in patients whose tumors are lymph-node positive.
This research was supported by a pilot grant in 2009 from Dr. Susan Love Research Foundation.

Keeping the focus on NAF, Gertraud Maskarinec, MD, PhD, professor at University of Hawaii Cancer Center in Honolulu, discussed her research exploring the effect of soy on NAF volume and composition. Studies suggest that soy food intake appears to lower breast cancer risk. In addition, NAF production is related to breast cancer risk. Dr. Maskarinec summarized findings from the field, including her dietary intervention study in premenopausal women on soy food intake, which did not find a relationship between soy and NAF characteristics. In conclusion, said Dr. Maskarinec, these NAF studies suggest there is no evidence that women who eat a moderate amount of soy have increased breast tissue activity, although there is some evidence that women with higher dietary fat and lactose intake may produce more NAF and that obesity may predict higher NAF volume.
Dr. Maskarinec received a pilot grant in 2005 for initial NAF research from Dr. Susan Love Research Foundation.

Seema A. Khan, MD, professor at Feinberg School of Medicine at Northwestern University in Chicago, moved the discussion from soy to processed soy isoflavone supplements. She

reported findings from a phase IIB randomized trial that investigated the effect of soy isoflavone supplements on breast epithelial proliferation and other biomarkers in 126 healthy women who were at high risk of developing breast cancer. The study used random fine needle aspiration (rFNA) to remove breast cells, which were examined for Ki-67 labeling index (Ki-67 LI). Overall, six months of soy isoflavone did not reduce breast epithelial proliferation, suggesting that these supplements would not be effective for breast cancer prevention. For premenopausal women, however, there were increases in the Ki-67 LI, which could suggest a possible adverse effect. Dr. Khan also discussed findings from the BEAM Study (Breast Estradiol and Methylation as Biomarkers of Breast Cancer Risk), which recruited through Dr. Susan Love Research Foundation's Army of Women. This study used rFNA to investigate whether a breast tissue DNA methylation profile they developed changed during the menstrual cycle or across menopause; and whether the DNA methylation profile correlated with mammographic density, cytomorphology or Gail risk. Dr. Khan reported that the gene promoter methylation panel provided information about risk that was independent of known risk markers, laying the groundwork for future research.

Consortium Contest

The formal proceedings were followed by presentations from 14 teams that formed during the Symposium to compete for pilot grants.

Public Presentation

The public presentation gave community members an opportunity to hear from Dr. Susan Love and four other Symposium participants: Dr. Paul J. Van Diest, advocate Ghecemy Lopez, Dr. Sheldon Feldman, and Dr. Sara Sukumar.

Each presenter discussed unique aspects of the Symposium, and the important opportunity it offers intraductal researchers to share their findings and advance the field as a whole. "Susan is the glue that keeps us together," said Dr. Van Diest. "Over the years, at these meetings, we've seen the field expand. Now we're seeing the use of new applications and new biomarkers that are putting us further along the path that could lead to new ways to diagnose breast cancer, new ways to treat it, and, maybe, even prevent it."

Dr. Feldman emphasized the sense of community and trust the Symposium engendered. "There is no other meeting where scientists, clinicians, and advocates are together for a few days sharing ideas, sharing unpublished data and there is no paranoia," he said. "Everyone wants to get published, but this an opportunity to share and collaborate."

Ghecemy Lopez emphasized the importance of what advocates give to the Symposium—and take away. "This is mind blowing and brain stimulation at a maximum level," she said. "To see the potential that we have right now encourages me to continue as an advocate and get patients involved in clinical trials."

Each presenter also discussed personal highlights. These ranged from the "organ on the chip" to the role of bacteria in the breast to the science that was presented on the analysis of a single cell. Also included were the scientific advances that have highlighted the subpopulations within a single tumor, and the systems biology that ties different fields together with deep knowledge across many disciplines.

Commenting from the audience, Judith Salerno, MD, president and CEO of Susan G. Komen, said, "I spent many years at the NIH funding safe science. It was energizing to be here and to see this research. I'm glad we could support it."

Consortium Grant Awards

At the close of the Symposium, Dr. Susan Love Research Foundation awarded a total of $50,000 in grants funded by Susan G. Komen to support multidisciplinary consortia formed at the Symposium. Grants were awarded to:

<u>Immune Profiling of Benign Breast Disease</u>

Sabina Adhikary, PhD, John Wayne Cancer Institute; Amy Degnim, MD, Mayo Clinic; Peter Sieling, PhD, John Wayne Cancer Institute; Michelle Rakoff and Lissa Levin, Advocates.

Dr. Sabina Adhikary is a research associate at the Laboratory of Translational Immunology at the John Wayne Cancer Institute, which explores the innate and adaptive immunity in breast cancer. Dr. Amy Degnim leads a team that uses the Mayo Clinic Benign Breast Cohort, which includes about 15,000 women to study whether premalignant changes in breast tissue can be used to predict an individual woman's breast cancer risk. This consortium received $16,000 to explore whether immune profiling of lymphocytes in women with benign breast disease can predict who will go on to develop breast cancer. This research could lead to the identification of new predictive markers for breast cancer risk and opportunities for prevention.

Optical Coherence Tomography for Breast

Sheldon M. Feldman, MD, Columbia University; Fatih Levent Balci, MD, Acibadem University (AU), Istanbul in affiliation with Columbia University; Christine P. Fleming, PhD, Columbia University; Linda Wilkes, Advocate.

Dr. Sheldon Feldman and Dr. Fatih Balci study intraductal approaches to breast cancer. They are currently investigating the effectiveness of optical coherence tomography (OCT), which is used in cardiology to look at blood vessels from the inside using light instead of sound (as an ultrasound does) to create three-dimensional images. OCT may be able to identify early changes in the breast duct before ductal carcinoma in situ (DCIS) or cancer forms. This consortium received $17,000 to study whether it is feasible to use OCT to distinguish different types of breast cells and tissues and whether OCT can show features of invasive cancers or DCIS that are detected on ultrasound or mammography. This work could identify reproducible ways to image early changes in the ducts and set the stage for prevention studies.

Intraductal Therapy & Prevention of Breast Cancer: De-Jamming JAM-A

Sara Sukumar, PhD, Johns Hopkins University School of Medicine (JHUSOM); Ann Hopkins, PhD, Royal College of Surgeons in Ireland; David Euhus, MD and Vered Stearns, MD, JHUSOM; Kim Wright, MS, Advocate.

Dr. Sara Sukumar studies the safety and effectiveness of intraductal therapy in animal models of breast cancer. Dr. Ann Hopkins has conducted research on a tight junction protein called Junctional Adhesion Molecule-A (JAM-A), which has been found to drive functional behaviors associated with breast cancer progression and directly regulate HER2 protein levels. The overexpression of JAM-A has been shown to be predictive of an aggressive tumor and a poor prognosis. Early preclinical data suggests a drug that targets JAM-A has the potential to be an effective breast cancer treatment. This consortium received $17,000 to analyze overexpression of JAM-A in ductal carcinoma in situ (DCIS); determine if this expression correlates with clinical factors; and investigate whether treatments delivered directly into the breast ducts could target JAMA-A and prevent or reduce DCIS in animal models of breast cancer. This research could lead to prevention of invasive breast cancer through a local means.

A fourth grant, funded by Atossa Genetics, was awarded to:

TLR5 Agonist-Antitumor Immunity

Peter Sieling, PhD, John Wayne Cancer Institute (JWCI); Sara Sukumar, PhD, Johns Hopkins University School of Medicine; Maggie DiNome, MD, JWCI; Lissa Levin and Michelle Rakoff, Advocates.

Peter Sieling is the assistant director of the Laboratory of Translational Immunology at the John Wayne Cancer Institute, where he studies the innate and adaptive immunity of breast tumors. Many breast tumors contain inflammatory cells, particularly CD8 lymphocytes. Patients with these tumor-infiltrating CD8 lymphocytes have been found to have a better outcome. Toll-like receptor 5 (TLR5) helps the immune system recognize specific pathogens. It also activates an innate antimicrobial immune response. Laboratory studies have shown that a TLR5 agonist, flagellin, inhibits the growth of breast cancer cells. Dr. Sara Sukumar's research has explored the safety and effectiveness of intraductal therapy in animal models of breast cancer. This consortium received $20,000 to study the safety and

effectiveness of intraductal flagellin in animal models, with the ultimate goal of conducting a clinical trial of intraductal flagellin in breast cancer patients. This may serve as a way to prevent invasive breast cancer from developing.

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