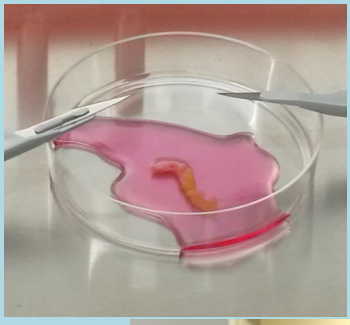
The Komen Tissue Bank White Paper



An Analysis of the Choice to Use Healthy, Normal Tissue as Controls in Breast Cancer Research

The Susan G. Komen® Tissue Bank at the IU Simon Cancer Center (Komen Tissue Bank, or KTB), part of Indiana University, is the only biorepository in the world containing breast tissue and blood products from women who do not show evidence of breast cancer at the time of donation. To more deeply understand the evolution of the disease, it is necessary to compare abnormal, cancerous tissue to normal, healthy tissue.

To date, more than 5,000 women have donated matched tissue and blood samples to the Komen Tissue Bank, and another 3,500 have contributed blood only. The KTB holds two to three breast tissue collection events each year, either in Indianapolis or in a different U.S. city based on diversity, and collects the following types of samples: fresh frozen tissue, formalin-fixed paraffin-embedded (FFPE) tissue, cryopreserved tissue; and blood products including whole blood, plasma, serum, and DNA from lymphocytes. KTB samples are collected, processed, and stored following stringent SOPs based on biorepository best practices. We are dedicated to following up with our donors by contacting them every year for updates to the rich medical history we have already compiled. This makes us not only a biorepository, but a longitudinal study.

Researchers wanting to acquire KTB samples should complete a Tissue Request Proposal Form and submit it on or before one of the four posted submission dates per year. To see what samples are available: use the Virtual Tissue Bank (VTB) to search the data, sort it into different order, and filter through the different categories. Samples are available for reasonable cost recovery fees. If you would like to simply use the VTB data rather than or in addition to requesting samples, all data is available to you for downloading at no cost. Researchers who have acquired and worked with samples from the KTB must agree to return raw data created from projects using those samples, and to appropriately attribute the acquisition of KTB data and samples in any published work. This data sharing hopefully minimizes duplication and hence accelerates research.

The Komen Tissue Bank is transforming breast cancer research by offering NORMAL, high-quality, richly annotated tissue samples to scientists worldwide. We are passionate about sharing our research resources. The KTB offers scientists an opportunity to participate in, and to influence, ongoing breast cancer treatment and prevention discovery.

by Katherine E. Ridley-Merriweather, MA
keridley@iupui.edu

INSIDE:

Abstract..... ..front cover
List of Figures 2
Introduction 2
The Komen Tissue Bank Offers a New Pathway to Treatments and a Cure..... 3
Virtual Tissue Bank 4
Cost Breakdowns 5
Case Study:
Mary Sehl, M.D., PhD 6-7
Conclusion ..... back cover
References .....back cover

The Susan G. Komen® Tissue Bank at the IU Simon Cancer Center
550 University Blvd.,
AOC Room 6042,
Indianapolis IN 46205
P: 866.763.0047
F: 317.274.6746
komentb@iupui.edu
www.komentissuebank.iu.edu

Introducing the Komen Tissue Bank

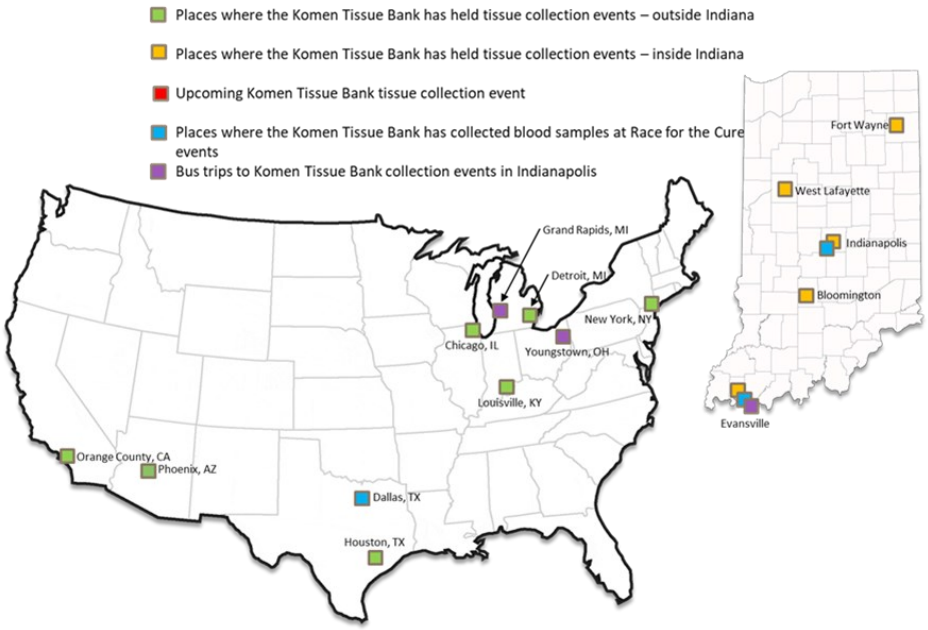


Figure 1. Komen Tissue Bank collection event locations

The Komen Tissue Bank represents a revolutionary approach to breast cancer research. It is the first and only biobank in the world collecting healthy breast tissue from women not known to have breast cancer, to be used as normal controls by researchers everywhere. By understanding how normal breast tissue looks and acts, scientists expect to pinpoint the changes that occur when cancer develops, thus speeding the discovery of a cure(s), and—ultimately—prevent breast cancer from happening at all.

Today, almost 5000 women—ranging in age from 18 to 92—have contributed healthy breast tissue to the Komen Tissue Bank. These samples have been used in research projects by investigators here in Indiana, as well as by nationally and internationally acclaimed scientists from U.S. and global institutions. For several years the KTB collected tissue only from women who lived in or were willing to travel to Indiana. Now we do the traveling, holding one event each calendar year in a different U.S. city chosen primarily for racial and ethnic diversity of population and availability of an appropriate medical partner (see Figure 1).

The one-of-a-kind Komen Tissue Bank provides researchers with an opportunity to help women battle back against a disease that affects far too many. Scientists who wish to request and use KTB tissue must have funding and IRB approval in place for their projects when they apply. To be approved to receive tissue samples, researchers must agree to share their data with the world through the KTB Virtual Tissue Bank, so others can build on their findings.

What are some questions the Komen Tissue Bank can help answer? This document will help breast cancer researchers learn about a resource that will enable them to approach their work differently. Some examples of studies being done on normal breast tissue samples:

How does the normal breast change in response to normal hormonal fluctuation?

How does the aging process change the breast?

How do breast cancer risk factors affect the breast on the molecular level?

What is the difference between the normal breast and the cancerous breast?

What are early signs of malignant transformation?

List of Figures

Figure 1.
Komen Tissue Bank collection event locations 2
Figure 2.
Number of KTB samples collected as of July 2018 4
Figure 3.
Cross-section of healthy breast tissue 4
Figure 4.
Komen Tissue Bank available sample types 5
Figure 5.
Percentages of KTB samples by race and ethnicity as of January 2017 5

The Komen Tissue Bank Offers a New Pathway to Treatments and a Cure

In their work examining the regulatory pathways present in the normal breast and the normal developmental workings that are threatened during early breast cancer progression, Hilton and Graham (2014) concluded, "In order to determine what is abnormal, we need to fully define what is normal.". Current research is showing it is time for a paradigm shift.

How can normal breast tissue be used to further and/or enhance your research? Because breast cancer is not always detected in a mammogram and can occur prior to screening at the age of 40 in the United States, normal tissue is key for developing better ways of assessing risk and detecting the disease in women and men. By identifying risk factors before cancer happens, scientists can develop more comprehensive strategies for prevention and treatment (Sherman et al., 2012). Studying normal breast tissue can provide information on non-genetic and genetic risk factors for breast cancer, uncover ways to mediate these risks before breast cancer happens, and allow scientists to create biomarker screenings that can be used in risk assessment and early detection (Sherman et al., 2012).

Researchers thought they were using "normal" tissue prior to the arrival of the Komen Tissue Bank. Was it truly normal, though? Before the existence of the KTB, tumor-adjacent tissue was considered normal, as was tissue from breast reductions and patients with benign breast disease. Research has now determined, however, that this is not the case. A team of researchers from the Mayo Clinic investigated 455 samples from the Komen Tissue Bank and compared them to samples from women who underwent breast reduction and from women who were part of the Mayo Clinic Benign Breast Disease Cohort Study. Degnim et al. (2012) wanted to compare these samples to see if normal tissue was indeed different. The Mayo scientists found that the normal tissue had significantly fewer histologic abnormalities than the other samples and represented a tissue resource consistent with lower breast cancer risk. These findings confirm that KTB tissue is a better representation of low risk, healthy tissue to use as a control (Degnim et al., 2012).

To understand the breadth and depth of research outcomes that are possible when using KTB healthy breast tissue samples as normal controls, it is helpful to look at work that has already been completed or is currently in progress:

- Researchers involved in a multi-institutional global investigation looked at why early full-term pregnancy is one of the most effective natural protections against breast cancer. Using multiple sample sets, including from the KTB, they found that women who have been pregnant have significant differences in their breast tissue and stem cell regulators, which are thought to be key sites for breast cancer development. Identifying these differences in women who have and have not experienced pregnancy may help in cancer risk assessment and prevention (Choudhury et al., 2013).
- Pardo et al. (2014) looked at how birth control and the menstrual cycle affect DNA expression in breast tissue. This represented the first-ever next-generation sequencing of 20 normal breast tissue specimens, and examined how gene expression was different depending on phases of the menstrual cycle and hormonal birth control use. They found that 255 genes were differentially expressed based on menstrual cycle phase, and that hormonal birth control did not significantly change gene expression.
- Women who are diagnosed after birth with breast cancer have significantly poorer outcomes, but it is not known why. During pregnancy and within 12 months of giving birth, women experience significant changes in their breasts, including an increase in tissue and ducts for milk production. In this study, Jindal et al. (2014) looked at tissue from premenopausal women who had never given birth, who were pregnant and lactating, and who had delivered a baby in a certain timespan. They found that within 12 months of giving birth, the breast completely morphed back to pre-pregnancy state and that 80-90% of the tissue grown in preparation for lactation was killed by the body. In addition, the body goes into a wound-healing process which triggers the immune system, and this may be linked to why breast cancer diagnosed during the postpartum window is so aggressive. By understanding this critical time of breast development, scientists may be able to provide therapies to women at a higher risk and identify prevention strategies.

For more information about using KTB samples, you can access the researcher section of our website here. Please click here to view an up-to-date list of more than 40 manuscripts of studies using or concerning KTB samples.

The Virtual Tissue Bank

The Virtual Tissue Bank, or VTB, is an important feature incorporated into the Komen Tissue Bank website. This completely free-of-cost, online resource is an immediately accessible way to share information about our samples with anyone interested in doing or supporting research. Biomedical and other researchers are able to access our samples with complete, de-identified annotation. Providing the VTB is one way the KTB incorporates our goal of limiting redundant research.

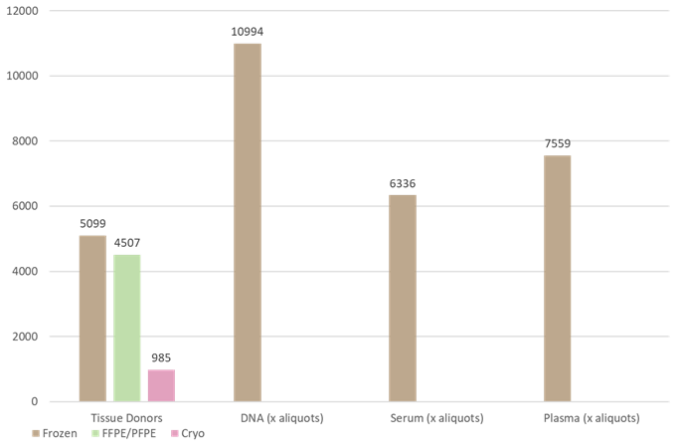


Figure 2. Number of KTB samples collected as of July 2018

How can this tool benefit you?

Through searching the VTB, researchers can know exactly what samples the KTB is holding and can determine how many of each type of sample we have collected, before thinking about submitting a sample request.

Here are actions a researcher can take when using our Virtual Tissue Bank *(see Figures 2, 3, & 5)*:

- Find experiment data from past research conducted with specific KTB samples, including links to manuscripts.
- Search sample availability using a variety of search fields including: age, BMI, race, menstrual status, breast cancer risk factors, pregnancies, comorbidities, hormone replacement therapy, etc.
- Find participant annotation data about specific barcodes, including: H&E images, mammograms, data generated from past experiments, and SNP data about race and ethnicity.
- Download image files including H&Es and mammograms.

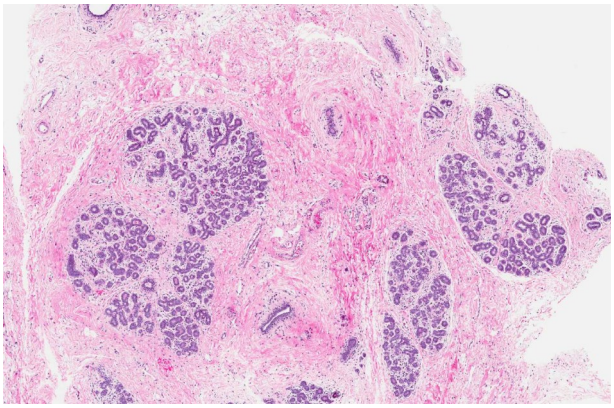


Figure 3. Cross-section of healthy breast tissue

<u>We request that if you download or make use of data from the VTB and use it in a manuscript please reference the KTB as follows</u>**:**

"Data from the Susan G. Komen Tissue Bank at the IU Simon Cancer Center were used in this study. We thank contributors, including Indiana University who collected data used in this study, as well as donors and their families, whose help and participation made this work possible."

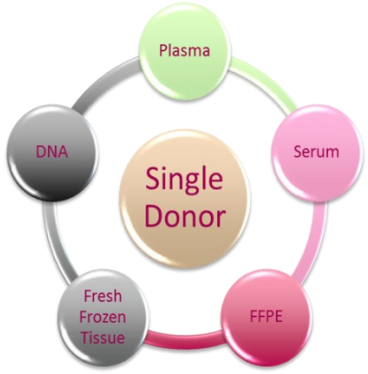
Cost Breakdowns

Cost Breakdowns

The Komen Tissue Bank is a non-profit organization that operates on the cost recovery schedule noted below; these figures reflect the cost of obtaining each type of sample (*see figure 4)*. This cost recovery schedule helps defray the cost associated with providing high quality samples to breast cancer researchers.

Sample Type	Cost /aliquot or section
Tissue – 125 cubic mm / aliquot	$225.00
Tissue sections – between 5-8 microns thick	$ 20.00
Blood products (plasma – 750 ul / aliquot, and serum – 600 ul / aliquot)	$ 55.00
DNA – min 15 micrograms /aliquot	$ 50.00

Service	Cost /hr
Additional work by KTB staff to obtain data from donors	$75



- Whole Blood
- DNA
- Fresh Frozen Breast Tissue
- Cryopreserved Breast Tissue
- FFPE blocks & H&E imag-

Figure 4. Komen Tissue Bank available sample types

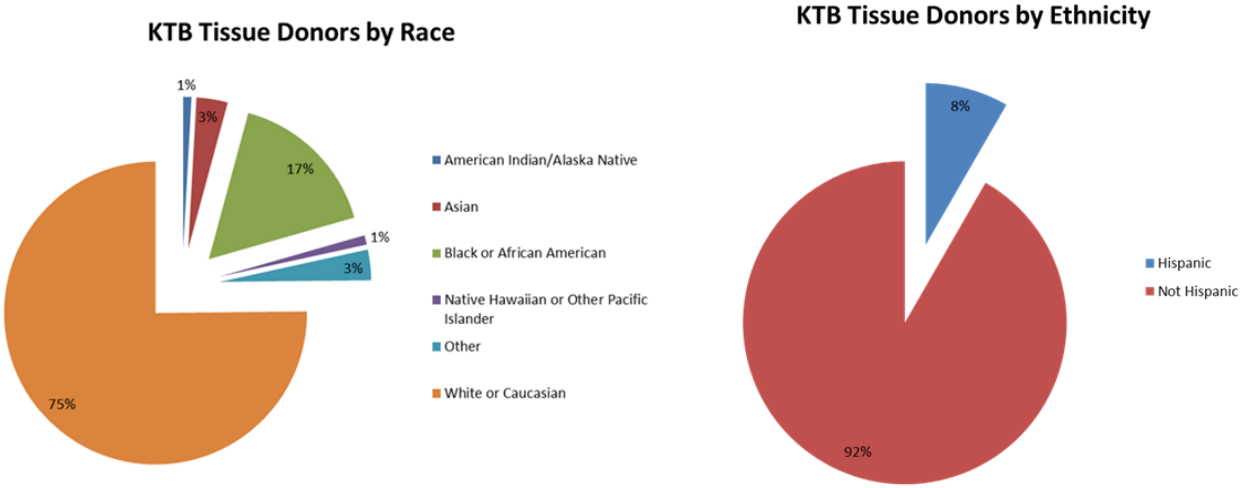


Figure 5. Percentages of KTB samples by race and ethnicity as of January 2017

Case Study:
Mary Sehl, M.D., PhD; UCLA David Geffen School of Medicine

Background: Dr. Mary Sehl is a Medical Oncologist and breast cancer researcher in the Division of Hematology-Oncology, Dept. of Medicine , and Associate Professor in the Dept. of Biomathematics at the David Geffen School of Medicine at UCLA. Dr. Sehl received her MD in 2001 from Brown University; after completing her internship and residency in Internal Medicine at the UCLA/VA Greater Los Angeles Healthcare System, she earned her PhD from UCLA in 2009. She joined the faculty in the Division of Hematology-Oncology in 2009 and has been actively engaged in translational research in cancer and aging, and breast cancer genetics and epigenetics.

In 2008 Dr. Sehl received a Young Investigator Award from the American Society of Clinical Oncology and in 2009 she accepted a Career Development Award from The ASCO Cancer Foundation and the Breast Cancer Research Foundation to study patterns and predictors of functional decline in early stage breast cancer survivors. In 2012 Dr. Sehl was awarded a UCLA KL2 Translational Scholar Award to develop a mathematical model of the dynamics of transitions in the breast cancer stem cell niche in collaboration with Dr. Max Wicha at the University of Michigan. Most recently, she received a pilot award from the Iris Cantor UCLA Women's Health Center and UCLA Clinical and Translational Science Institute to collaborate with the Komen Tissue Bank. Dr. Sehl has co-authored over 30 publications in peer-reviewed medical journals, as well as three book chapters, and is a member of the Patient and Survivors Program in the UCLA Jonsson Comprehensive Cancer Center.

The Challenge: Dr. Sehl had submitted a research proposal to the KTB seeking a very specific cohort of samples: women who had donated tissue and blood on at least two different time points. This table defines additional requirements for the breakdown by parity and menopausal status for this cohort of 100 donors.

	Parous	Nulliparous
Premenopausal	25	25
Postmenopausal	25	25

Further, Dr. Sehl's project required specific medical history annotation for each sample. The Komen Tissue Bank is unique in its acquisition of rich annotation for its samples.

In Dr. Sehl's words: *My experience working together with the Komen Tissue Bank has been outstanding in so many ways. Everything is done with the highest level of professionalism. Responses are prompt and thorough, and conference calls are easily facilitated and always helpful. The quality of the samples and data received are excellent. The staff are always ready to answer questions and help link researchers with any resources needed.*

This project required the following data: age, race/ethnicity, Ashkenazi, tobacco/alcohol use, education, cancer history, prophylactic surgeries, lactation history, HRT, family history of breast/ovarian cancer, and BRCA1/BRCA2 status.

The KTB worked closely with Dr. Sehl to identify and ship the samples that met the cohort criteria.

Case Study: continued

In Dr. Sehl's words: *"Preliminary studies of epigenetic aging markers in breast tissue revealed accelerated biologic aging in breast. However, these preliminary studies were done using normal adjacent tissue from patients affected by breast cancer. We aimed to examine whether breast tissue in healthy women exhibited accelerated aging using epigenetic markers, and to compare breast and blood tissue from matched samples in healthy donors.*

While it is easy to obtain data from peripheral blood samples, it is rare to find breast tissue biopsy material from healthy women.

My experience working with the Komen Tissue Bank far exceeded my expectations. The specimens and data are of high quality, and the survey data are extensive and well annotated. Everything arrived in a timely manner and the researchers and staff are always willing to help facilitate progress and collaboration.

I had heard of this tremendous resource several years ago at a breast cancer meeting, and learned that it was being developed to advance research in normal breast biology, which is absolutely essential for understanding what goes awry in breast carcinogenesis. Recently, when my research team became interested in questions of whether breast tissue ages faster than other tissues in the body, I began inquiring what samples were available from different tissues at different time points."

Once Dr. Sehl's project was approved, she worked with KTB staff to identify the appropriate samples for her research. The KTB works collaboratively to be sure the samples fit the needs of both the researcher(s) and the research project. It can be a very iterative process involving communication between the KTB staff and the researcher who is receiving the samples. The KTB has a variety of sample types including fresh frozen, cryopreserved FFPE, DNA, plasma, serum, and matched tissue/ blood.

In Dr. Sehl's words: *Using the KTB samples, we were able to directly compare DNA methylation levels of breast and blood tissues from within the same individual, and found that the breast appears to age faster than peripheral blood. We plan to extend our study to examine what factors (e.g. total menstrual years, number of pregnancies, breast feeding) influence the epigenetic age of breast tissue. We have further developed a collaboration with Dr. Natascia Marino in the KTB to examine methylation patterns in breast tissue that are associated with risk of later developing breast cancer.*

We are able to estimate the biologic age of a tissue based on DNA methylation levels at 353 sites. We find that epigenetic age is elevated in healthy female breast tissue compared with peripheral blood from the same individual. We find that this difference is more dramatic in younger women, and diminishes as one approaches the age of the menopausal transition. We hypothesize that this difference is caused by exposure to estrogen and chronic cell cycling.

I was amazed by the wealth of longitudinal specimens and survey data available, and also by how collaborative, knowledgeable, and helpful the KTB team was.

We anticipate that epigenetic patterns in breast tissue that may provide additional information in identifying women at high risk for developing breast cancer. Ultimately, this information has the potential to influence screening and prevention.

Two measures of service I value are promptness and quality control. Looking at time from request for samples/ variables to time delivered is a measure where the KTB is outstanding. This feature is very important for driving the progress of the project and meeting grant and publication deadlines to move the research forward. When I performed QC measurements on the data I received it was clear that the specimens had been handled with vigilance and the data were managed carefully.

Dr. Sehl has been very pleased with the samples that were identified for her project. Her manuscript based on the work she did with these samples was published in Breast Cancer Research and Treatment in 2017.

Click here to read Dr. Sehl's resulting publication from her collaboration with the KTB.

Conclusion

Fueled by the progression of breast cancer research, medical management of the disease has shown significant improvement over the last several years (Curtis et al., 2012). Much of that progress has come about through the focused study of breast cancer oncogenesis. The Komen Tissue Bank is a one-of-a-kind biorepository storing breast tissue and blood from healthy women with no sign of breast cancer. If your research is focused on the source or prevention of this disease, it is worth your while to investigate how using KTB samples could add value to your work. Requesting and receiving KTB samples is clearly explained in the Researcher section of our website.

Research has shown that normal tissue collected by the Komen Tissue Bank is a valuable control, showing significantly fewer abnormalities than the other samples previously described as normal. In addition, other important research is defined in the more than 40 published manuscripts (to date) of studies using healthy Komen Tissue Bank tissue samples. These studies report findings such as:

- Normal tissue from the KTB had considerably fewer abnormalities than samples of tissue previously deemed to be "normal";
- Normal tissue and tissue adjacent to the tumor had many significant differences;
- Within 12 months of giving birth the breast completely morphed back to pre-pregnancy state, and 80-90% of tissue grown in preparation for lactation was killed by the body;
- 255 genes were differently expressed based on menstrual cycle phase, and birth control did not significantly change gene expression;
- Higher levels of certain hormones in premenopausal and postmenopausal women were linked with higher duct counts, suggesting that hormones may influence breast cancer risk by delaying duct atrophy and involution.

Scientists who have chosen to use the Komen Tissue Bank to provide NORMAL samples for their research controls have expressed great satisfaction. The samples are richly and deeply annotated, of great quality, and reasonably priced. The KTB website is well organized, helpfully designed, and the proposal process is simple and well laid out. We invite you to join those researchers who have already discovered us.

To initiate a discussion with the Komen Tissue Bank, or for more information, please contact Jill Henry, Chief Operating Officer, jihenry@iupui,edu or 317-278-2829.

References

Choudhury, S., Almendro, V., Merino, V. F., Wu, Z., Maruyama, R., Su, Y., . . . Kowalczyk, A. (2013). Molecular profiling of human mammary gland links breast cancer risk to a p27+ cell population with progenitor characteristics. *Cell stem cell, 13*(1), 117-130.

Curtis, C., Shah, S. P., Chin, S.-F., Turashvili, G., Rueda, O. M., Dunning, M. J., . . . Yuan, Y. (2012). The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature, 486*(7403), 346.

Degnim, A. C., Visscher, D. W., Hoskin, T. L., Frost, M. H., Vierkant, R. A., Vachon, C. M., . . . Hartmann, L. C. (2012). Histologic findings in normal breast tissues: comparison to reduction mammaplasty and benign breast disease tissues. *Breast cancer research and treatment, 133*(1), 169-177.

Hilton, H. N., & Graham, J. D. (2014). The molecular landscape of the normal human breast–defining normal. In: BioMed Central.

Jindal, S., Gao, D., Bell, P., Albrektsen, G., Edgerton, S. M., Ambrosone, C. B., ... & Schedin, P. (2014). Postpartum breast involution reveals regression of secretory lobules mediated by tissue-remodeling. Breast Cancer Research, 16(2), R31.

Pardo, I., Lillemoe, H. A., Blosser, R. J., Choi, M., Sauder, C. A., Doxey, D. K., . . . Atale, R. (2014). Next-generation transcriptome sequencing of the premenopausal breast epithelium using specimens from a normal human breast tissue bank. *Breast cancer research, 16*(2), R26.

Sauder, C. A., Koziel, J. E., Choi, M., Fox, M. J., Grimes, B. R., Badve, S., . . . Vaughan, M. B. (2014). Phenotypic plasticity in normal breast derived epithelial cells. *BMC cell biology, 15*(1), 20.

Sherman, M. E., Figueroa, J. D., Henry, J. E., Clare, S. E., Rufenbarger, C., & Storniolo, A. M. (2012). The Susan G. Komen for the Cure Tissue Bank at the IU Simon Cancer Center: A unique resource for defining the "molecular