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Frontiers in Oncology, 2017; 7:289-1-289-4

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Originally published at:
http://doi.org/10.3389/fonc.2017.00289
Editorial: How Reproductive History Influences Our Breast Cancer Risk

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Reproductive history has profound effects on women's breast cancer (BCa) risk. With fertility rates falling, the age of childbearing increasing, and the age of menarche decreasing each decade, it is critically important that we define the biological pathways linking reproductive history to BCa risk. In this special series on reproductive history and breast cancer risk, we hear from six groups who have expertise in this area and share their thoughts on the most pressing questions in the field.

Dall and Britt have an interest in the effects of hormones and reproductive events (menarche, parity, menopause) on breast cancer risk. They describe the increased risk of breast cancer in nulliparous nuns and discuss the current studies attempting to define the role of the mammary stem cells, ER+ cells, and growth factors in parity-induced protection (1, 2). In contrast to the decreased risk that comes with pregnancy, a woman's risk of ER+ BCa increases if she is older when she enters menopause (3, 4). Polymorphisms in the ER gene, ER signaling (5, 6), DNA damage/repair, and FSH and immune components (7–10) have been associated with the age at menopause but together still only explain a small portion of the timing. As mice do not undergo a natural menopause, experimental data on menopause and breast cancer are lacking. The other reproductive time point to influence cancer risk is the age at menarche; a younger age at menarche increasing BCa risk. This is worrying as the age of menarche has declined from 16.5 years in the 1800s, 13.5 in the early 1900s, to 12 years today. Biologically, menarche begins in response to rising circulating hormones including estrogen, and elevated estrogens have been found in girls experiencing precocious menarche (11). Despite this, genome-wide sequencing studies failed to find a strong association with estrogen-regulated genes (9, 12, 13). Hormone replacement therapy and oral contraceptive pill use both increased BCa risk in current users and can have more detrimental effects on younger users (14, 15). Together, this work highlights that the young breast is particularly sensitive to hormonal changes.

Atashgarian and colleagues study the influence of the reproductive cycle on immune cells within the breast and how this might relate to the increased risk of BCa in women who cycle for extended periods. They explain the effects of fluctuating estrogen and progesterone (during menstrual cycling) on the mammary epithelial cells, immune cells, and extracellular matrix. The ability of transformed cells to evade the immune response is a hallmark of cancer (16). Fluctuations in hormones during the mouse estrous cycle alters the abundance, phenotype, and function of local macrophages (17, 18), which can affect their ability to recognize DNA-damaged cells, phagocytose them, and generate adaptive immune responses (18). In particular, progesterone regulates the Th1/Th2 phenotypes of T cells in the mammary gland (19) and induces Th2 cytokines during pregnancy (20). Th1 cytokines are thought to mediate antitumor immunity and tumor rejection, whereas Th2 cytokines are produced by tumors and are involved in pro-tumorigenic responses (21, 22). Estradiol induces a pro-inflammatory cytokine profile in the estrus phase that can be mitigated by progesterone during

Keywords: estrogen, breast cancer risk, pregnancy, parity, lymphangiogenesis, obesity
other phases of the cycle (23). It is possible that aberrant hormo-
nal exposure can negatively influence the inflammatory milieu of
the breast and aid in tumor development and cancer progression.

Katz and her team have been trying to understand the role
of insulin-like growth factors (IGF) in parity-induced protection
against breast cancer. They dissected mammary glands from
parous mice and age-matched virgins and found Igf1r to be
hypermethylated and silenced in parous mammary glands (2)
aligning with the fact that high IGF1 levels are associated with
increased BCa risk (24, 25). Parous mice, with a low tumor inci-
dence (16%) compared to nulliparous mice (100%) treated with
carcinogen, had their protective effect eliminated if they were first
treated with IGF (83%) (26). This work is supported by a recent
DNA methylation study performed on parous and nulliparous
women showing that the IGF acid labile subunit (responsible
for transport of IGF1 in the circulation) was hypomethylated
with parity (27). Unfortunately, targeting the IGF pathway using
IGF1R inhibitors leads to toxic effects that will limit their use as
a preventative (28–30); however, this work highlights the impor-
tance of exploring other options to promote the parity-associated
breast cancer protection.

While there is a lot of interest in trying to define the role of
hormones in parity-induced protection, prior to the pro-
tection instilled by parity, each woman passes through a transient
increased risk period. Borges and colleagues have been studying
this postpartum period of mammary gland involution, character-
ized by regression and remodeling of the epithelium. Postpartum
BCa is diagnosed within 5 years of a woman’s most recent child-
birth (31, 32), and these cancers tend to be of poor prognosis with
an increased likelihood to metastasize (31–34). Similarly, tumor
cells implanted into postpartum murine hosts have increased
growth, invasive and metastatic capacities compared to those
implanted in nulliparous controls (35–39). This environment is

driven by immunosuppression and lymphangiogenesis and, here,
Borges and colleagues review the role for lymphangiogenesis,
the outgrowth of new lymphatic vessels (40–42). New lymphatic
formation or neo-lymphangiogenesis occurs in adult tissues in
response to infection, inflammation, and wound healing and is
stimulated by vascular endothelial growth factors within the dam-
aged tissue. The growth factors VEGF-A, -C, and -D produced by
the local fibroblasts, inflammatory cells, and macrophages bind
to their receptors on nearby lymphatic endothelial cells and cause
the lymphatics to expand (43–47). Borges et al. have shown that
neo-lymphangiogenesis occurs during postpartum mammary
gland along as does an upregulation of VEGF-C, VEGF-D, and
their receptors (36). This increase in lymphatic vessel density in
the postpartum breast (1–5 years post pregnancy) may explain
the increased likelihood of tumor development in these women
who make up a large proportion of all BCa diagnosed in young
(<45 years) Caucasian women (33).

Au and colleagues work on the role of adipose-derived estro-
gen in breast cancer development. Local estrogen production
(through the enzyme aromatase) is increased in tumor-bearing
breast tissue and systemic antiestrogen therapies are widely used
to target this in ER+ BCa patients. These hormonally driven
BCa are more prevalent in postmenopausal women where an
increased BMI has been associated with increased local breast
estrogen (48–50). In these women, the adipose tissue (rather
than the ovaries) is the primary site of estrogen production (51).
Aromatase is increased in breast adipose tissue of obese women
as a number of obesity-associated factors (inflammatory mediator
PGE2 and the adipokine leptin) can stimulate aromatase expres-
sion (52, 53). Au and colleagues have tried to find a factor that
inhibits aromatase expression in the adipose as a novel targeted
antiestrogen therapy. They found that the gut-derived hormone
ghrelin (produced in the stomach to regulate appetite and growth
hormone release) inhibits aromatase expression in adipose cells
(54, 55). In addition, circulating levels of ghrelin, and its una-
cetylated form des-acyl ghrelin, are lower in obese women and
cannot function as well to inhibit estrogen-driven BCa growth.
They describe the work they are now doing on Ghrerin/des-acyl
ghrelin mimetics as alternative endocrine therapeutics.

In order to define how aggressive a tumor is and possibly
identify if it would be responsive to endocrine therapy, clinics are
adopting gene-expression profiling on the tumors to provide an
intrinsic, molecular portrait of the tumor and identify the likeli-
hood of recurrence. Bernhardt and colleagues are concerned that
these tests have been developed using tumor tissue from
postmenopausal women (non-cycling) but are being used for
women at all ages. Of the gene expression tests available (PAM50,
Oncoype DX and EndoPredict) were developed in postmeno-
pausal women and now not performing well in premenopausal
women who make up 25% of all patients (56–58). It is postulated
that this is because the tests are largely reliant on proliferation and
estrogen signaling genes, which are expected to differ markedly
in pre- and postmenopausal women. The former undergo cyclical
production of ovarian hormones which drive proliferation,
differentiation, and apoptosis through gene expression changes
(59, 60). These hormonally driven changes are not present in
the postmenopausal women. Supporting these concerns, it has
been found that the MammaPrint gene expression test that was
developed and validated in women under 55 years of age (61, 62)
is performing well for younger women. This suggests that for the
most success in guiding clinical treatment, gene-expression tests
should be developed in the women for which we intend to use
them.

AUTHOR CONTRIBUTIONS

RA: this author assisted in the design and was involved in review-
ing articles. Assisted with drafting the editorial and approved
final version. WI: this author wrote two review articles and was
involved in reviewing articles. She assisted with drafting the
editorial and approved final version. KB: this author was the lead
editor and conceived the special issue. Invited all contributors,
rote a review article, and assisted with reviewing articles. Wrote
the editorial.

ACKNOWLEDGMENTS

RA was supported by an NBCF grant PS-15-022 fellowship. WI
was supported by an NBCF and THR Fellowship. KB was sup-
ported by an NBCF grant PS-16-021 fellowship and an NHMRC
new investigator grant.
REFERENCES

1. Dall G, Risbridger G, Britt K. Mammary stem cells and parity-induced breast cancer protection – new insights. *J Steroid Biochem Mol Biol* (2017) 170:54–60. doi:10.1016/j.jsbmb.2016.02.018

2. Katz TA, Liao SG, Petrucci VI, Dearth RK, Pathiraja TN, Hua Z, et al. Targeted DNA methylation screen in the mouse mammary genome reveals a parity-induced hypermethylation of Igf1r that persists long after parturition. *Cancer Prev Res (Phila)* (2015) 8(10):1000–9. doi:10.1158/1940-6207.CAPR-15-0178

3. Collaborative Group on Hormonal Factors in Breast Cancer. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol* (2012) 13(11):1141–51. doi:10.1016/S1470-2045(12)70425-4

4. Sisti JS, Collins LC, Beck AH, Tamimi RM, Rosner BA, Eliassen AH. Reproductive risk factors in relation to molecular subtypes of breast cancer: results from the nurses’ health studies. *Int J Cancer* (2016) 138(10):2346–56. doi:10.1002/ijc.29968

5. He LN, Xiong DH, Liu YJ, Zhang F, Recker RR, Deng HW. Association study of the oestrogen signalling pathway genes in relation to age at natural menopause. *J Cancer* (2007) 86(3):269–76. doi:10.1007/s12041-007-0034-7

6. Weel AE, Uitterlinden AG, Westendorp IC, Burger H, Schuit SC, Hofman A, et al. Estrogen receptor polymorphism predicts the onset of natural and surgical menopause. *J Clin Endocrinol Metab* (1999) 84(9):3146–50. doi:10.1210/jc.84.9.3146

7. He C, Kraft P, Chasman DI, Buring JE, Chen C, Hankinson SE, et al. Genetic loci at chromosomes 13, 19 and 20 influence age at natural menopause. *Hum Genet* (2010) 128(5):515–27. doi:10.1007/s00439-010-0878-4

8. Perry JR, Hsu YH, Chasman DI, Johnson AD, Albrecht E, et al. DNA mismatch repair gene MSH6 implicated in determining age at natural menopause. *Hum Mol Genet* (2014) 23(9):2490–7. doi:10.1093/hmg/ddt620

9. Stolk L, Perry JR, Chasman DI, He C, Mangino M, Sulem P, et al. Meta-analyses of DNA mismatch repair gene MSH6 implicated in determining age at natural menopause. *Hum Mol Genet* (2014) 23(9):2490–7. doi:10.1093/hmg/ddt620

10. Perry JR, Hsu YH, Chasman DI, Johnson AD, Elks C, Albrecht E, et al. Menopause. *Hum Mol Genet* (2014) 23(9):2490–7. doi:10.1093/hmg/ddt620

11. Ursin G, Ross RK, Sullivan-Halley J, Hanisch R, Henderson B, Bernstein L, et al. Use of oral contraceptives and risk of breast cancer in young women. *Breast* (2003) 362(9382):348–54. doi:10.1016/S0140-6736(03)14065-2

12. Rijnkels M, Kabotyanski E, Montazer-Torbati MB, Hue Beauvais C, Vassetzky Y, Rosen JM, et al. The epigenetic landscape of mammary gland development and functional differentiation. *J Mammary Gland Biol Neoplasia* (2010) 15(1):85–100. doi:10.1007/s10911-010-9170-4

13. Chua AC, Hodson LJ, Moldenhauer LM, Robertson SA, Ingman WV. Dual functions for macrophages in ovarian cycle-associated development and remodeling of the mammary gland epithelium. *Development* (2010) 137(24):4229–38. doi:10.1242/dev.059261

14. Wu MH, Chou YC, Chou WY, Hsu GC, Chu CH, Yu CP, et al. Relationships between critical period of estrogen exposure and circulating levels of insulin-like growth factor-I (IGF-I) in breast cancer: evidence from a case-control study. *Int J Cancer* (2010) 126(2):508–14. doi:10.1002/ijc.24722

15. Thordarson G, Shusher N, Leong H, Ochoa D, Rajkumar L, Guzman R, et al. Insulin-like growth factor (IGF)-I blunts the pregnancy-associated protection against mammary carcinogenesis in rats: evidence that IGF-I enhances cancer progression through estrogen receptor-alpha activation via the mitogen-activated protein kinase pathway. *Breast Cancer Res* (2004) 6(4):R423–36. doi:10.1186/bcr812

16. Borden EC, Triolo SP, Tomczak LM, Egan MA, Hecht GM, Kruger JO, et al. Use of oral contraceptives and risk of breast cancer in young women. *Breast Cancer Res Treat* (2011) 124(9):3901–12. doi:10.1007/s10593-011-0987-6

17. Anderson et al. Reproduction and Breast Cancer
38. Martinson HA, Jindal S, Durand-Rougey C, Borges VF, Schedin P. Wound healing-like immune program facilitates postpartum mammary gland involution and tumor progression. *Int J Cancer* (2015) 136(8):1803–13. doi:10.1002/ijc.29181
39. Stanford JC, Young C, Hicks D, Owens P, Williams A, Vaught DB, et al. Efferocytosis produces a prometastatic landscape during postpartum mammary gland involution. *J Clin Invest* (2014) 124(11):4737–52. doi:10.1172/JCI76375
40. Betterman KL, Harvey NL. The lymphatic vasculature: development and role in shaping immunity. *Immunol Rev* (2016) 271(1):276–92. doi:10.1111/imr.12413
41. Schulte-Merkel S, Sabine A, Petrova TV. Lymphatic vascular morphogenesis in development, physiology, and disease. *J Cell Biol* (2011) 193(4):607–18. doi:10.1083/jcb.201012094
42. Tammela T, Alitalo K. Lymphangiogenesis: molecular mechanisms and future promise. *Cell* (2010) 140(4):460–76. doi:10.1016/j.cell.2010.01.045
43. Cao Y, Linden P, Farnebo J, Cao R, Eriksson A, Kumar V, et al. Vascular endothelial growth factor C induces angiogenesis in vivo. *Proc Natl Acad Sci U S A* (1998) 95(24):14389–94. doi:10.1073/pnas.95.24.14389
44. Gallego E, Vicioso L, Alvarez M, Hierro I, Perez-Villa I, Blanes A, et al. Stromal expression of vascular endothelial growth factor C is relevant to predict sentinel lymph node status in melanomas. *Virchows Arch* (2011) 458(5):621–30. doi:10.1007/s00428-011-1044-7
45. Karkkainen MJ, Haiko P, Sainio K, Partanen J, Taipale J, Petrova TV, et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. *Nat Immunol* (2004) 5(1):74–80. doi:10.1038/ni1013
46. Klutz L, Norman S, Vieira JM, Masters M, Rohling M, Dubé KN, et al. Cardiac lymphatics are heterogeneous in origin and respond to injury. *Nature* (2015) 522(7534):62–7. doi:10.1038/nature14483
47. Schoppmann SF, Fenzl A, Nagy K, Unger S, Bayer G, Geleff S, et al. VEGF-C expressing tumor-associated macrophages in lymph node positive breast cancer: impact on lymphangiogenesis and survival. *Surgery* (2006) 139(6):839–46. doi:10.1016/j.surg.2005.12.008
48. Boyapati SM, Shu XD, Gao YT, Dai Q, Yu H, Cheng JR, et al. Correlation of blood levels in postmenopausal women. *Obesity (Silver Spring)* (2006) 14(9):1662–77. doi:10.1038/oby.2006.191
49. Lorincz AM, Sukumar S. Molecular links between obesity and breast cancer. *Endocr Relat Cancer* (2006) 13(2):279–92. doi:10.1677/erc.0.01279
50. Brown KA, McNees KJ, Hunger NI, Oakhill JS, Steinberg GR, Simpson ER. Subcellular localization of cyclic AMP-responsive element binding protein-regulated transcription coactivator 2 provides a link between obesity and breast cancer in postmenopausal women. *Cancer Res* (2009) 69(13):5392–9. doi:10.1158/0008-5472.CAN-09-0108
51. Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Transcriptional regulation of CYP19 gene (aromatase) expression in adipose stromal cells in primary culture. *Steroid Biochem Mol Biol* (1997) 61(3–6):203–10. doi:10.1016/ S0960-7660(97)80013-1
52. Au CC, Docanto MM, Zahid H, Raffaelli FM, Ferrero RL, Furness JR, et al. Des-acetyl ghrelin inhibits the capacity of macrophages to stimulate the expression of aromatase in breast adipose stromal cells. *J Steroid Biochem Mol Biol* (2017) 170:49–53. doi:10.1016/j.jsbmb.2016.07.005
53. Docanto MM, Yang F, Callaghan B, Au CC, Ragavan R, Wang X, et al. Ghrelin and des-acetyl ghrelin inhibit aromatase expression and activity in human adipose stromal cells: suppression of CAMP as a possible mechanism. *Breast Cancer Res Treat* (2014) 147(1):193–201. doi:10.1007/s10549-014-2869-2

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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