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Pregnancy associated breast cancer and its prognosis studied through gene expression analysis

Author: Adrienne Jones
Major: Microbiology and Cellular & Molecular Biology
Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

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The impact of pregnancy on the biology of cancer remains a poor area of prognosis for pregnant women. Interactions with diagnosing at age, stage, and grade remain unknown, regardless of the precision medicine and early diagnosis of such disorders. Breast cancer, specifically diagnosed during or 5-10 years post-childbirth, has fewer studies and poorer outcomes than non-pregnant control patients. It has also been found in epidemiological studies that pregnancy has shown a bidirectional, time-dependent effect on the risk of breast cancer. The rise of these cancers and how their genetic makeup differs from non-pregnant related breast cancer is unknown. It is known, compared to age-matched non-pregnancy-related breast cancers, PABC (pregnancy-associated breast cancer) and PPBC (postpartum breast cancer) has been characterized by an increased tumor incidence and aggressive nature. Determining whether pregnancy is related to a change in gene expression causing breast cancer and whether these changes are persistent and worse than non-PABC cancers are key predictors for disease prognosis. Also examining these changes, studies have searched for tumor patterns, gene expression changes, and genome sequencing data to find signatures for the molecular characteristics of PABC or PPBC.

Introduction

Breast cancer is the one of the most frequently encountered cancers in women, but the impact of pregnancy on the biology of cancer remains a far less studied area of study (1-13). Pregnancy-associated breast cancer occurs in approximately one in 3000 pregnancies (2). Interactions with diagnosing at age, stage, and grade remain unknown, regardless of the precision medicine and early diagnosis of such disorders (1-4,6,7,10). Breast cancer diagnosed during pregnancy, also referred to as pregnancy-associated breast cancer (PABC), is commonly defined as breast cancer diagnosed during or within one year of pregnancy (1,2,5,6,9,11). In contrast, postpartum breast cancer (PPBC) is diagnosed within two to ten years of childbirth and has an observed increase in breast cancer risk that peaks at about five-seven years post-delivery (3,4,6,11). PABC and PPBC are associated with cancer in younger women (<45 years) and account for a decent percent of all new breast cancer incidents in many developing countries (1-4,7,10,11). Breast cancer diagnosed during or after pregnancy has been observed as more aggressive and is related to poorer prognosis than non-pregnancy-related breast cancer (1-4,5,9,13). The rise of these cancers and how their

genetic makeup differs from non-pregnant related breast cancer is unknown. PABC and PPBC have been recognized in other studies as being more aggressive because of occurrence in a younger population, a general more advanced T stage at the time of diagnosis, higher grade tumors, and generally negative estrogen receptor state (1-4,7,8,10). The study and understanding of these patients will further clinical interest on to how to treat PABC/PPBC patients compared to non-pregnant breast cancer patients. Insights into the biology of PABC and PPBC suggest new findings regarding parity in disease pathogenesis. Recent studies have searched for various diagnostic biomarkers, genomic differences, and epigenetic changes to explain the worse outcomes of PABC and PPBC (1-4). Also, all studies reviewed in the current writing compared cohorts of non-pregnancy related breast cancers to PABC or PPBC patients and identified characteristics matched to an aggressive histopathologic profile or genetic expression as predictors of poor outcome. Studies also either controlled for the differences in clinicopathological features or compared biologically similar age-matched patients when comparing non-pregnant and pregnancy related breast cancers. Studies going further are a mix of large population-based cohorts or limited low patient cohorts. Molecular profiles, histopathologic profiles, and genetic patterns all show clues about the mechanisms for the aggressive nature of pregnancy on increased breast cancer risk (1-5,9,12,13).

Recent Progress

Investigating the impact of pregnancy on the effects of breast cancer by comparing PABC patients and non-pregnant controls has not extensively been studied using copy number alternations in whole genome sequencing data but was done a small cohort in (3). Pregnancy causes a series of cellular and molecular changes in mammary epithelial cells (MECs) of female adults (8,13). In addition, pregnancy can also modify the predisposition of rodent and human MECs to initiate oncogenesis (3,8,13).

The PABC/PPBC patients were demonstrated with different tumor CN patterns to demonstrate more in-depth genetic disease progression. This team of researchers started first with examining tumor tissue blocks, the tissue that is found that is diseased or abdominal to be then further examined under a microscope (8). Examining these tumor tissue blocks allowed them to detect 20 known oncogenes and 2 tumor suppressor genes for copy number alterations that differed from non-PBC cancer patients (3).

RNA expression data is mainly used for gene studies, so the study (3) provides clinical implications for PPBC and PABC patient treatment. A recent study (2) tried to observe a difference in gene expression changes between pregnant and non-pregnant women by investigating the common occurrence of estrogen receptor positive markers in breast cancer and found that PPBC patients' poor prognostic phenotypes when ER+.

Many of these studies have brought forward new biomarkers, such as free circulating nucleic acids, the detection of epigenetic changes, apoptotic bodies, and even the gene expressions of oncosomes, genes with the potential to cause cancer (1,4,6). These factors have been found due to specific protein expressions, DNAs, and mRNAs.

Discussion

With our known literature (11) and updated knowledge to this date, (6) emphasize of breast cancer in relation to pregnancy has been slowly growing. In the oldest study used in this review (1) expands our known background information about the recent advances in pregnancy related breast cancer. In relation to the aspect of time, breast tissue had not been studied in relation to gene expression and quantification of such genes. Using real time quantitative PCR and mammoplasty or breast biopsies cells captured by laser microdissection the expression of targeted genes sets could be studied. Targeted

genes sets (64 genes in total) included angiogenesis, ECM remodeling, inflammation, and hormone signaling. The cohort of women for the study consisted of similarly aged women all either being nulliparous, recently pregnant (0-2 years since birth); or postpartum (2-10 years). Of the total genes selected, 14 were found that were significantly expressed between the two groups (non-pregnancy related and pregnancy related breast cancer) (1). Of the genes found, inflammation related genes were the most strongly discriminating. Expression profiles of hormone signaling genes were also differential, providing evidence for the first time that pregnancy is associated with changes in breast tissue and cancer risk. It was also shown that pregnancy had a lasting effect by not discriminating in the upregulation of the found inflammation genes of recently pregnant breast cancer cases and postpartum breast cancers.

The total 22% of the selected genes in the study found to be significant and the biological correlation of each gene's expression provide a strong conclusion for the evidence of altered gene expression of pregnancy related breast that is significantly different than non-pregnancy related breast cancer. The aggressive nature of pregnancy related breast cancer can be speculated in part to the contributed genes found in the study. The first data of its kind provided a strong foundation for future studies that can continue to describe the parity relationship pregnancy has on breast cancer and the ways it can be studied at the gene level.

The following study (3) shows how the investigation of pregnancy on breast cancer has grown since the (1) study. A comparative analysis using whole genome sequencing data from a cohort of PABC and non-PABC patients was done with a total of 167 patients (57 were diagnosed at pregnancy). The team wanted to specifically find unique molecular characteristics between the two groups that significantly aim at characterizing PABC breast cancer tumor biology. Genes also known as drivers in breast cancer (such as MYC and TP53) were examined as well to identify any copy number alterations and gene expression change. Of the two groups, copy number profiles showed no significant differences. When non-silent mutations were examined PABC cases had a significantly higher number of mutations compared to controls (20 vs. 12) and of these TP53 and PIK2CA were the most frequent. A higher frequency of missense mutation in mucin gene families were also observed in PABC than non 45.7% vs. 11.1%). PABC also showed a higher occurrence of base-substitution signatures 1 and 20, but no clinicopathological features associated with signatures were observed in PABC patients. Sig20-positive patients were shown to have a worse prognosis compared to PABC Sig20 patients and controls. This is hypothesized because of the mismatch repair deficiency of Sig20. Unique PABC biology and the features shown in this research may be key factors when promoting tumor during pregnancy. Unlike other studies up until this point, the molecular characteristics found in higher frequency in pregnancy related tumors suggest new routes of clinical interest. Genetic analysis while is expanding has yet to be compared alongside histopathological profiles to assess discriminating phenotypes.

In the study (4) with the largest cohort study of PABC patients (n=741) to age matched non-PABC patients (n=741), all patients came from one country's demographic profile. As observed in additional studies, PABC patients often times have more tumors and tumors of a higher grade (grade I: 1.5% vs. 12.4%, grade

II: 16.9% vs. 31.3% and grade III: 80.3% vs. 39.5%) (4). Of their observed tumors in both PABC and non-PABC patients, PABC tumors were often less ER-receptor positive, 38.9% vs. 68.2% (4). Interestingly this study also observed a majority of PABC patients were diagnosed during pregnancy (74.2%) with nearly half of these occurring during the third trimester (47.3%) (4). Of the patients in the study, they also included patients that terminated their pregnancies, patients that were both lactating, not

breastfeeding, but only included patients diagnosed within 6 months postpartum. The large nationwide study results were in line with smaller comparable studies in PABC cohorts cited within the studies literature. Although the mentioned studies within this study cited a more aggressive histopathologic profile for younger women breast cancer, their PABC patients still showed an even more aggressive histopathologic profile regardless. A limitation of the study is that data is more a discussion from pathology reports, and therefore overall survival analyses (OS) were unable to be performed. The study larger focuses more on the overview of the histopathologic profiles of these patients and serves more as a starting point for further research. The large conclusion for this survey is the significantly higher proportion of high-grade, ER – tumors among PABC patient.

The higher proportion of ER- tumors in PABC patients seemed opposite to the generally higher levels of estrogen and progesterone levels observed during pregnancy. The higher portion of ER- tumors is also in contrast to young breast cancer cases where the majority of cases are ER+, >65% (2). They concluded these tumors are most likely being driven by other growth factors or other hormones, and not estrogen/mammary cells themselves and cite supported literature where they observe this findings. Further in-depth research unraveling the genetic background of PABC patients and the observed more aggressive histopathological profile can be observed in the study (2). The high mortality of breast cancer was explored in ER+ nulliparous (NPBC) and ER+ PPBC young breast cancer patients to further knowledge of the molecular distinct aspects by which the two cancer types differ. Breast tissue was only taken from a total of 16 matched PPBC (9 patients) and NPBC (7 patients) ER+. The goal of identifying these differences of non-pregnancy related breast and pregnancy related cancer is less conclusive and thus probably due to the low number of patients in the cohort.

P53 cell cycle, immune system, and related genes were shown to be expressed at different levels between PPBC and non-PPBC cancers also are differentiated based also on ER + cases. RNA sequencing and rank based gene set enrichment analysis was performed to investigate gene expression. Pronounced signature for T-cell presence/activation, reduced ER signaling, and increased cell cycle genes were found for ER+ PPBC cases. The researchers suggest the pronounced T-cell presence in PPBC samples is caused by a transient event of normal mammary gland involution leading to the influence on breast cancer biology.

The signatures found for ER+ PPBC cases were shown to have a significantly lower survival rate compared to low expressing cases. When applied to a large population of young women with breast cancer, distinct signatures for ER+ PPBC and transcription factors regulons showed a significant overall effect on the lethality of PPBC.

The observed loss of the wildtype TP53 in PPBC tumors was also hypothesized to reflect a breast-feeding induced involution biology. As established in other studies cited in this study, P53 has been a physiological regulator of involution in the secretory epithelium tissue. Reduced downstream ER signaling in PPBC cases was interpreted as being more analogous with ER-negative disease and an adaptation of the involution state of postpartum tissue. In sum, poor prognosis in PPBC is also demonstrated to have gene signatures associated with involution of mammary glands. All of these studies have allowed the foundations of further studies to build the molecular investigation of the impact of pregnancy on breast cancer biology.

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