Mammographic density and epithelial histopathologic markers

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Abstract

Background: We explored the association of mammographic density, a breast cancer risk factor, with hormonal and proliferation markers in benign tissue from tumor blocks of pre-and postmenopausal breast cancer cases.

Methods: Breast cancer cases were recruited from a case-control study on breast density. Mammographic density was assessed on digitized prediagnostic mammograms using a computer-assisted method. For 279 participants of the original study, we obtained tumor blocks and prepared tissue microarrays (TMA), but benign tissue cores were only available for 159 women. The TMAs were immunostained for estrogen receptor alpha (ERα) and beta (ERβ), progesterone receptor (PR), HER2/neu, Ki-67, and Proliferating Cell Nuclear Antigen (PCNA). We applied general linear models to compute breast density according to marker expression.

Results: A substantial proportion of the samples were in the low or no staining categories. None of the results was statistically significant, but women with PR and ERβ staining had 3.4% and 2.4% higher percent density. The respective values for Caucasians were 5.7% and 11.6% but less in Japanese women (3.5% and -1.1%). Percent density was 3.4% higher in women with any Ki-67 staining and 2.2% in those with positive PCNA staining.

Conclusion: This study detected little evidence for an association between mammographic density and expression of steroid receptors and proliferation markers in breast tissue, but it illustrated the problems of locating tumor blocks and benign breast tissue samples for epidemiologic research. Given the suggestive findings, future studies examining estrogen effects in tissue, cell proliferation, and density in the breast may be informative.

Background

Although a vast body of literature describes a positive association between mammographic density and breast cancer risk with an estimated relative risk of 4 or higher for women in the highest as compared to the lowest density category [1], not much is known about the underlying histopathology of breast density [2]. Such knowledge may contribute to breast cancer prevention because it may
improve our understanding of the relation between density and breast cancer risk as well as the potential for risk prediction and modification. The two types of tissue that give rise to radiologically dense breasts are epithelium and stroma forming the microenvironment of epithelial cells which constitute less than 5% of breast tissue [3,4]. The main component of stromal tissue is collagen [5]. It was hypothesized that the extent of mammographic density is proportional to the amount of breast epithelium and that the higher breast cancer risk associated with breast density is due to a larger number of glandular cells at risk for malignant transformation [6,7]. This idea is supported by findings of an association between the proliferation of stroma, epithelium, or both with breast density in subjects with breast abnormalities [8,9]. Unfortunately, research in healthy women is limited to forensic studies [10,11] and one study of breast reduction samples [5]. In breast cancer patients, increased amounts of collagen were associated with breast density in several reports [5,9,11,12], while the results on cell proliferation were mixed [5,13,14].

As risk factors that induce cell proliferation [15,16], endogenous sex steroids and hormone therapy (HT) are associated with higher breast cancer risk [17,18]. Whereas HT, in particular estrogen plus progestogen therapy, increases mammographic density [19], the relation between endogenous sex steroids and mammographic density is less clear. One study observed an association with endogenous estrogens [20] but others did not [21-23]. As breast tissue levels are partly determined by estrogen production in adipose tissue, breast size as marker for adipose tissue surrounding the epithelial cells may possibly be a better marker for tissue levels than circulating estrogen levels. Endogenous progesterone was found to be related to mammographic density in one report among premenopausal women [24]. The biological activities of endogenous and exogenous estrogens on breast tissue are mediated by nuclear estrogen receptors (ER) α and β. Differential effects of ERα and ERβ are of interest because ERβ appears to be more antiproliferative while ERα has proliferative activity [25,26]. Progesterone, an ovarian steroidal hormone, acts through its specific receptor (PR) [27,28]; PR expression has been shown to be a sensitive indicator of estrogenic effects in cells [29].

To understand how hormone receptors and cell proliferation are related to breast density, this study examined the expression of ERα, ERβ, and PR as well as HER2, Ki-67, and Proliferating Cell Nuclear Antigen (PCNA) [30], in relation to mammographic density among breast cancer patients with Caucasian, Japanese, and Hawaiian ethnicity. We are convinced that these associations are best studied in benign breast tissue and, thus, restricted this analysis to breast cancer patients for whom benign tissue samples placed on tissue microarrays (TMA) were available. The relation between mammographic appearance of the breast and marker expression in tumor tissue is a separate issue that needs further study [31].

**Methods**

**Study population**

The study was approved by the Institutional Review Boards of the University of Hawaii and Wake Forest University; all subjects provided informed consent in writing. We recruited subjects for the TMA study from 607 breast cancer cases who had participated in the Multiethnic Cohort (MEC) [32] and a nested case-control (NCC) study of mammographic densities [33]. Of these, 177 women were excluded because their tumor blocks were not available from the Hawaii Tumor Registry (HTR). Recruitment letters and questionnaires were mailed to the remaining 430 subjects; 323 (75%) women returned the consent forms. Another 12 women were deceased but linked to the HTR and could, thus, be included in the study. For 279 out of these 335 subjects, pathologic blocks from breast cancer surgery were located and used to create TMAs; no tissue from prior benign biopsies was available. At entry into the MEC, all participants had completed a questionnaire that inquired about demographics, reproductive behavior, anthropometric measures, and family history of breast cancer [32]. As part of the NCC, women completed an additional one-page breast health questionnaire that asked about previous breast surgery, menopausal status, mammography history, and HT use [33].

**Tumor microarrays**

TMAs were prepared according to standard procedures [34,35]. In brief, a surgical pathologist (JK) identified blocks from a given patient with sufficient tissue. For each of these blocks a single hematoxylin and eosin (H&E) slide was prepared on which the same pathologist marked representative areas of malignant and benign tissue. The H&E slide was aligned with the corresponding “donor” block and a 0.6 mm cylindrical tissue specimen was taken from the selected area and transferred to a “recipient” paraffin block using a tissue-arraying instrument (Beecher Instruments, Sun Prairie, WI). When available, four malignant cores and four benign cores per patient were placed in one of six blocks. Out of the 2,232 cores to be placed (four malignant and four benign samples for 279 women), tissue was insufficient for 12% of malignant and 29% of benign specimens resulting in 1,773 tissue samples (79.4%) for analysis. At least one benign or malignant core was available for 268 women. Several 5 μm sections were mailed to Wake Forest University for immunohistochemical staining.

**Immunohistochemistry and pathologic evaluation**

The TMAs were stained for the following markers: ERα, ERβ, PR, and PCNA (Clones 6F11, EMR02, 1A6, and PC10, respectively; all from Novocastra Labs, Newcastle-
Mammographic density assessment

Breast density readings used for the present study had all been obtained previously as part of the NCC study [33]. All mammographic films were scanned with a Kodak LS85 Film Digitizer (absorbance range, 0.001–4.1; Eastman Kodak Company, Rochester, New York) at a resolution of 98 pixels per inch. One of the authors (GM) performed computer-assisted density assessment with the Cumulus package [37]. After the reader determined a threshold for the edge of the breast and the dense tissue, the computer computed the number of pixels that constituted the total and the dense area and the ratio between the two, i.e., percent breast density. To convert the pixels of the area into cm², a factor of 0.000676 was used. The intraclass correlation coefficients (ICC) to assess reliability were 0.96 (95% confidence interval (CI): 0.95, 0.97) and 0.996 (95% CI: 0.995, 0.997) for the size of the dense and the total breast area, respectively. This resulted in an ICC of 0.974 for percent density (95% CI: 0.968, 0.978). For the present study, the cranial caudal view closest to, but before, breast cancer diagnosis was selected; the mean time between the two dates was 10.0 ± 14.8 months.

Statistical analysis

SAS statistical software package version 9.1 was used for all analyses (SAS Institute Inc., Cary, NC). The dense breast area was square root transformed to normalize the distribution. For ease of interpretation, back transformed mean values are presented. For all histology markers, the mean percentage of stained cells of all available cores per sample was calculated. To assess marker agreement by subject, ICCs were computed. For all six markers, the distributions of samples were skewed with strong left tails. The interquartile ranges were 0.0–8.1%, 0.0–13.8%, 0.0–1.5%, 0.0–14.5%, 0.0–0.6%, 0.8–16.2% for ERα, ERβ, PR, HER2, Ki-67, and PCNA, respectively. Therefore, samples were divided into two categories; negative staining (<10% of cells stained) and positive staining (≥10% of cells). Because the number of women with positive staining for PR and Ki-67 was very low (4 and 13 respectively), we dichotomized the results into no vs. any epithelial staining. Linear regression models were used to analyze the association between histological markers as categorical variables and mammographic density (absolute density and percent density) as continuous variables. Associations were adjusted for variables previously shown to be associated with mammographic density including age at mammogram, body mass index (BMI), ethnicity, age at menarche, parity, age at first live birth, HT use at mammogram, menopausal status, and family history of breast cancer. Separate analyses stratified by ethnicity (Caucasian and Japanese) and by total size of the breast with the median as cutpoint were also performed.

Results

Women in the TMA study were slightly younger, were more likely premenopausal, and had a lower BMI than in the original NCC study, but were otherwise similar (Table 1). The study population included 49 Caucasians, 70 Japanese, 21 Native Hawaiians, and 19 women of other ethnicities. Mean total breast area as measured on the mammograms was largest for Caucasian women and smallest for Japanese women (135 and 91 cm², respectively). BMI and total breast area were strongly correlated 0.52 (p < 0.0001). Absolute mammographic density was
Table 1: Characteristics of women recruited for the TMA study and the original study*

| Variable                  | Original study | TMA study |
|---------------------------|----------------|-----------|
| Sample size (N)           | 607            | 159       |
| Ethnicity (%)             |                |           |
| Caucasian                 | 185 (30.5)     | 49 (30.8) |
| Hawaiian                  | 80 (13.2)      | 21 (13.2) |
| Japanese                  | 287 (47.3)     | 70 (44.0) |
| Other                     | 55 (9.1)       | 19 (12.0) |
| Age at mammogram          |                |           |
| < 13 years                | 324 (54.4)     | 86 (54.1) |
| 13–14 years               | 217 (36.4)     | 86 (53.5) |
| > 14 years                | 55 (9.2)       | 19 (12.0) |
| Number of children (%)    |                |           |
| 0–1                       | 172 (28.3)     | 43 (27.0) |
| 2 to 3                    | 312 (51.4)     | 86 (54.1) |
| > 3                       | 123 (20.3)     | 30 (18.9) |
| Age at first live birth (%)|                |           |
| < 21 years                | 80 (13.6)      | 21 (13.2) |
| 21–30 years               | 359 (61.0)     | 102 (64.2) |
| > 30 years                | 56 (9.5)       | 11 (6.9)  |
| N/A                       | 94 (16.0)      | 25 (15.7) |
| HT use at mammogram (%)   |                |           |
| No use                    | 264 (43.5)     | 64 (40.3) |
| Estrogen only             | 174 (28.7)     | 46 (28.9) |
| Estrogen plus progestogen | 169 (27.8)     | 49 (30.8) |
| Breast measures           |                |           |
| Total breast area         | 117.9 ± 58.1   | 110 ± 52.9|
| Breast density in percent | 35.3 ± 23.3    | 38.4 ± 24.8|
| Absolute density          | 35.9 ± 27.0    | 39.5 ± 23.4|
| Menopausal Status (%)     |                |           |
| Premenopausal             | 152 (25.0)     | 60 (37.7) |
| Postmenopausal            | 455 (75.0)     | 99 (62.3) |

* Means ± standard deviation unless stated otherwise.

highest in Caucasian women and in the subgroup of women with other ethnicities (43 cm²) and lower in Hawaiian (36 cm²) and Japanese women (34 cm²). Percent mammographic density was highest among the subgroup of women with other ethnicities (50%) and lowest in Hawaiian women (30%), while it was intermediate in Japanese (40%) and Caucasians (38%).

A substantial proportion of the samples were in the low or no staining categories (Table 2). The percentages were 88%, 70%, 49%, 67%, 42%, and 60% for ERα, ERβ, PR, HER2, PCNA, and Ki-67, respectively. A similar proportion of Japanese and Caucasian women were in the highest staining category for all markers; none of the differences was statistically significant. The sample size was too small to examine other ethnic groups. Small differences in breast density were seen between staining categories of several markers, but, with two exceptions in the subgroup of Caucasians, none of the results were statistically significant. Percent density was higher in the overall and stratified analyses for subjects with PR staining (all women: 3.4%; Caucasian: 5.8%; Japanese: 3.5%). In women with higher ERβ staining, percent density was higher in the total population and in Caucasians (2.4% and 11.6%) but not in Japanese. No associations of percent density with ERα and HER2/neu were observed. Percent density was somewhat higher in women with Ki-67 staining, both in the overall population (3.4%) and in Caucasians (3.8%) and Japanese (4.4%). Positive PCNA staining showed slightly higher percent density in all women and in Japanese but not in Caucasians.

As an exploratory analysis, we stratified by total breast area to capture possible effects due to high adiposity. More women with large breasts had PR expression than women with small breasts (58% vs. 44%, p = 0.08) (Table 3). The opposite was seen for ER expression (ERα: 62% vs. 77%, p = 0.44; ERβ: 56% vs. 65%, p = 0.30). Women with large breasts and positive staining for all markers, except Ki-67, had higher percent densities, especially for PR (6.2%), ERβ (6.4%), and PCNA (4%). Although not statistically significant, women with small breasts who stained for hormonal markers showed slightly lower percent density except for PR with 4.3% higher density in category 2. Those with positive Ki-67 staining had 4.4% higher density, whereas positive staining for PCNA made no difference among women with small breasts. With few exceptions, the associations with absolute area were similar to the findings with percent density. Restricting the analyses to postmenopausal women did not change the results; no significant associations were observed (data not shown).

Discussion

This investigation of breast density and immunohistochemical marker expression in TMAs observed no significant associations in the entire study population, but it appeared that mammographic density was slightly higher for women with PR expression as compared to those with no PR expression. This observation was consistent across the two major ethnic groups and women with different breast sizes. The difference between low and high categories was 3–4% in density which, if a true finding, may translate into a 6–8% higher breast cancer risk [33]. Only in women with large breasts, mammographic density was slightly higher in subjects with ERα, ERβ, and HER2 expression, but again, the results were not statistically significant. For category 2 expression of Ki-67 and PCNA, percent breast density was slightly higher in the entire population. The findings in Caucasians who, on average, have larger breasts than Japanese largely reflected the results in the subgroup of women with large breasts, whereas the findings in Japanese women tended to be closer to the results of women with small breasts. The
higher percent density among women with Japanese ancestry despite their lower breast cancer risk was also observed in the original study [33]. It is due to the small breast sizes that result in a higher percent of the breast occupied by dense tissue. As shown in cross-sectional comparisons, the size of the dense area appears to be a better indicator of risk when different ethnic groups are compared [38,39].

Six previous reports examined the underlying histological markers for breast density; one study used forensic breast samples [11], one examined reduction mammoplasty samples [5], one collected fine needle biopsies [13], two used tissues surrounding non-cancerous breast lesions [9,12], and another one identified non-cancerous tissue from mastectomy specimens [14]. Of these six studies, only one study assessed ER$\alpha$ and PR expression and did not find an association, but the sample size was only 56 [14]. Three studies looked at Ki-67 expression; one found no association with mammographic density [13], one found a positive association [14], and one described less proliferation in dense areas [5]. As far as we know, no previous results for ER$\beta$, HER2/neu, and PCNA have been reported.

The observation that in women with small breasts, percent density was slightly lower for those with higher ER expression, whereas it was higher in women with large breasts and positive ER expression suggests that a possible effect of ER expression on breast density, if it exists, may depend on the amount of local estrogens. Apart from ovarian production, estrogens are metabolized from androgens in adipose tissue [40]. Thus, in women with large breasts, tissue estrogen levels would also be higher due to the larger amount of fat tissue. This idea agrees with a report that women with a nipple aspirate fluid (NAF) phenotype characterized by higher BMI and percentage body fat had higher NAF estrogen levels [41]. Therefore,
the higher PR expression in women with large breasts as compared to women with small breasts may reflect responsiveness to estrogen [29]. Despite the non-significant results, these observations may indicate that higher ER expression in combination with high tissue levels of estrogens influence breast density.

This epidemiologic investigation took advantage of the TMA approach that allows assessment of marker expression in a large number of pathologic samples under similar staining conditions [34,42]. Another benefit of TMAs was that multiple markers could be analyzed using sections of the TMA block without exhausting the material. However, a disadvantage of the method is the loss of samples during immunostaining due to lack of adhesion to glass slides and cross-sectional variation in the amount of epithelial tissue. The use of cores with a larger diameter may alleviate that problem. Alternatively, immunohistochemistry could be performed on full sections, if they are available. Another issue with TMAs is that consecutive sections may reveal different types of tissue for the same core due to heterogeneity with tissue depth. As shown before, benign tissue samples were more affected than malignant cores [42,43]. Since a maximum of 4 benign core sections per woman was placed, information on a substantial number of women was obtained. Despite identifying benign specimens for only 159 out of 279 subjects due to the difficulties with benign tissue in the tumor blocks, little evidence for selection bias was detected (Table 1). Obviously benign biopsies would be the preferred source of material for this type of research because benign tissue adjacent to tumor tissue may be different from benign tissue in the other breast or in breast tissue of women without cancer. Unfortunately, that type of tissue is not easy to obtain. The amount of material available from stereotactic biopsies is often too small for TMA preparation and women with benign biopsies cannot be identified through tumor registries. Although breast reduction surgery would yield large amounts of tissue, women undergoing that procedure represent a selected subgroup with

Table 3: Marker expression and mammographic density stratified by total breast size

| Marker       | Small total breast area | Large total breast area |
|--------------|-------------------------|-------------------------|
|              | Category*               | P-value                 | Category              | P-value                 |
|              | 1           | 2                     | 1           | 2                     |
| ERα         | % density†  | 41.3                  | 38.0                  | 0.55                  | 30.7                  | 33.7                  | 0.61                  |
|             | Dense area# | 29.5                  | 29.1                  | 0.92                  | 34.6                  | 39.7                  | 0.53                  |
|             | Number      | 63                    | 16                    | 59                    | 19                    |
| ERβ         | % density   | 41.3                  | 39.5                  | 0.71                  | 29.9                  | 36.3                  | 0.24                  |
|             | Dense area  | 28.9                  | 29.7                  | 0.82                  | 34.1                  | 41.2                  | 0.34                  |
|             | Number      | 50                    | 29                    | 60                    | 18                    |
| PR          | % density   | 39.0                  | 43.3                  | 0.36                  | 27.1                  | 33.3                  | 0.19                  |
|             | Dense area  | 28.8                  | 29.8                  | 0.78                  | 28.3                  | 40.0                  | 0.05                  |
|             | Number      | 45                    | 36                    | 32                    | 45                    |
| HER2/neu    | % density   | 41.7                  | 39.8                  | 0.71                  | 30.4                  | 33.5                  | 0.51                  |
|             | Dense area  | 30.3                  | 28.6                  | 0.65                  | 35.2                  | 37.6                  | 0.71                  |
|             | Number      | 55                    | 24                    | 49                    | 28                    |
| Ki-67       | % density   | 38.2                  | 42.6                  | 0.35                  | 29.7                  | 30.9                  | 0.81                  |
|             | Dense area  | 25.4                  | 31.8                  | 0.07                  | 35.8                  | 34.5                  | 0.85                  |
|             | Number      | 30                    | 49                    | 35                    | 41                    |
| PCNA        | % density   | 41.0                  | 39.9                  | 0.81                  | 29.9                  | 33.9                  | 0.43                  |
|             | Dense area  | 29.4                  | 28.8                  | 0.86                  | 33.7                  | 40.5                  | 0.32                  |
|             | Number      | 43                    | 38                    | 52                    | 25                    |

* Categories for ERα, ERβ, HER2/neu and PCNA; category 1 <10% staining, category 2 ≥ 10% staining. Categories for PR and Ki-67; category 1 = no staining, category 2 = any staining
† Mean percent breast density
# Mean dense area in cm²
Mean values and p-values calculated using general linear models adjusted for age at mammogram, BMI, ethnicity, HT use at mammogram, menopausal status, parity, age at first live birth, age at menarche and family history of breast cancer.

(ERα = estrogen receptor alpha; ERβ = estrogen receptor beta; PR = progesterone receptor; HER2/neu = Human Epidermal growth factor receptor2; PCNA = Proliferating Cell Nuclear Antigen; BMI = body mass index)
predominantly fatty breast tissue and different mammographic patterns [44].

Conclusion
This investigation illustrates the problems of obtaining benign breast tissue samples for a representative sample of participants in epidemiologic studies. To benefit from the advantages of the TMA technique in future research, it is recommended to select the areas for taking the tissue cores carefully and to minimize the loss of specimens during immunostaining. Based on our few suggestive findings, future mammographic density investigations may pursue estrogenic effects as assessed by PR and cell proliferation using Ki67 and other such markers to examine the question of local estrogen activity in relation to breast density. The slightly higher breast density with PR expression suggests a stronger estrogenic response in mammographically dense breasts possibly leading to stronger cell proliferation, an idea supported by associations of breast density with hyperplasia and other benign breast pathology [8,10,45,46]. Although not statistically significant, the higher breast density with steroid receptor expression in women with large breasts as compared to women with small breasts indicates a role of locally produced estrogens in women with more adipose tissue [40]. On the other hand, it is most likely that chance was responsible for the small differences observed in this exploratory study. Given the weak associations in the current investigations and the discrepant findings across studies, the roles of growth factors and the properties of the stromal matrix in shaping breast density also deserve further attention [4,9,12].

List of abbreviations
BMI: Body mass index; CI: Confidence interval; ER: Estrogen receptor; HT: Hormone therapy; HTP: Hawaii tumor registry; ICC: Intraclass correlation coefficient; MEC: Multietnic cohort; NAF: Nipple aspirating fluid; NCC: Nested case control study; PCNA: Proliferating Cell Nuclear Antigen; PR: Progesterone receptor; TMA: Tissue microarray.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MV carried out the statistical analysis and drafted the manuscript. GM conceived of the study, obtained funding, and directed the statistical analysis. EL participated in the design and the data collection and contributed to the statistical analysis. JSS contributed to the data collection and the statistical analysis. JK evaluated and selected the pathologic specimens and provided input on pathologic issues. BH provided access to the pathologic specimens and provided input on pathologic issues. JMC performed the pathologic evaluation of the TMAs and contributed to the study design. GM conceived of the study, obtained funding, and contributed to the study design. JMC performed the pathologic evaluation of the TMAs and contributed to the study design. All authors read and approved the final manuscript.

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References
1. McCormack VA, dos Santos Silva I: Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006; 15:1159-1169.
2. Boyd NF, Rommens JM, Vogt K, Lee Y, Hopper JL, Yaffe MJ, Paterson AD: Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol 2005; 6:798-808.
3. Boyd NF, Jensen HM, Cooke G, Han HL: Relationship between mammographic and histological risk factors for breast cancer. J Natl Cancer Inst 1992; 84:1170-1179.
4. Warren R, Lakhani SR: Can the stroma provide the clue to the cellular basis for mammographic density? Breast Cancer Res 2003; 5:225-227.
5. Hawes D, Downey S, Pearce CL, Bartow S, Wan P, Pile MC, Wu AH: Dense breast stromal tissue shows greatly increased concentration of breast epithelium but no increase in its proliferative activity. Breast Cancer Res 2006; 8:B24.
6. Albanes D, Winick M: Are cell number and cell proliferation risk factors for cancer? J Natl Cancer Inst 1988; 80:772-774.
7. Trichopoulos D, Lipman RD: Mammary gland mass and breast cancer risk. Epidemiology 1992; 3:523-526.
8. Byrne C, Schairer C, Brinton LA, Wolfe J, Parekh N, Salame M, Carter C, Hoover R: Effects of mammographic density and benign breast disease on breast cancer risk (United States). Cancer Causes Control 2001; 12:103-110.
9. Alowami S, Troup S, Al-Haddad S, Kirkpatrick I, Watson PH: Mammographic density is related to stroma and stromal proteoglycan expression. Breast Cancer Res 2003; 5:R129-R135.
10. Bartow SA, Pathak DR, Mettler FA, Key CR, Pile MC: Breast mammographic pattern: a concatenation of confounding and breast cancer risk factors. Am J Epidemiol 1995; 142:813-819.
11. Li T, Sun L, Miller N, Nicklee T, Woo J, Hulse-Smith L, Tsao MS, Khokha R, Martin L, Boyd N: The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer. Cancer Epidemiol Biomarkers Prev 2005; 14:343-349.
12. Guo YP, Martin LJ, Hanna W, Banerjee D, Miller N, Fishell E, Khokha R, Boyd NF: Growth factors and stromal matrix proteins associated with mammographic densities. Cancer Epidemiol Biomarkers Prev 2001; 10:243-248.
13. Khan QJ, Kimler BF, O’dea AP, Zalles CM, Sharma P, Fabian CJ: Mammographic density does not correlate with Ki-67 expression or cytology in benign breast cells obtained by random periareolar fine needle aspiration from women at high risk for breast cancer. Breast Cancer Res 2007; 9:R35.
14. Harvey JA, Santen RJ, Petroni GR, Boobjerg VE, Smolkin ME, Sherriff FS, Russel J: Histologic changes in the breast with menopausal hormone therapy use: correlation with breast density, estrogen receptor, progesterone receptor, and proliferation indices. Menopause 2008; 15:67-73.
15. Sutherland RL, Prall OW, Watts CK, Musgrove EA: Estrogen and progestin regulation of cell cycle progression. J Mammary Gland Biol Neoplasia 1998; 3:63-72.
16. Doineau-Skou SF, Sergio CM, Carroll JS, Hui R, Musgrove EA, Sutherland RL: Estrogen and antiestrogen regulation of cell cycle progression in breast cancer cells. Endocr Relat Cancer 2003; 10:179-186.
The Endogenous Hormones and Breast Cancer Collaborative Group: Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002, 94:606-616.

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, et al.: Risks and benefits of estrogen plus progesterin in healthy postmenopausal women: principal results from The Women's Health Initiative randomized controlled trial. JAMA 2002, 288:321-333.

Greendale GA, Rebossi BA, Slone S, Wasilauskas C, Pike MC, Ursin G: Postmenopausal hormone therapy and change in mammographic density. J Natl Cancer Inst 2003, 95:30-37.

Greendale GA, Palla SL, Ursin G, Laughlin GA, Crandall C, Pike MC, Rebossou BA: The association of endogenous sex steroids and sex steroid binding proteins with mammographic density: results from the Postmenopausal Estrogen/Progestin Interventions in Mammographic Density Study. Am J Epidemiol 2005, 162:826-834.

Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:876-882.

Tamimi RM, Byrne C, Colditz GA, Hankinson SE: Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2007, 99:1178-1187.

Verheus M, Peeters PH, Van Noord PA, Schouw YT van der, Grobbee DE, van den Brandt PA: No relationship between circulating levels of sex steroids and mammographic breast density: the Prospective EPIC cohort. Breast Cancer Res 2007, 9:R53.

Noh JJ, Maskarinec G, Pagano I, Cheung LW, Stanczyk FZ: Mammographic densities and circulating hormones: a cross-sectional study in premenopausal women. Breast 2006, 15:20-28.

Barkthem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson JA, Nilsson S: Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists. Mol Pharmacol 1998, 54:105-112.

Fox MH, Davis RJ, Shupnik MA: ERbeta in breast cancer: onlooker, passive player, or active protector? Steroids 2008, 73:1039-1051.

Gao X, Loggie BW, Nawaz Z: The roles of sex steroid receptor promoters and enhancers in breast cancer. Mol Cancer 2002, 1:7.

Gao X, Nawaz Z: Progesterone receptors – animal models and cell signaling in breast cancer: Role of steroid receptor coactivators and corepressors of progesterone receptors in breast cancer. Breast Cancer Res 2002, 4:182-186.

Hurni LL, Byrne C, Colditz GA, Johnson SE: Endogenous breast cancer in postmenopausal women: reappraisal of nine prospective studies. J Natl Cancer Inst 2002, 94:606-616.

Lundell AM, Byrne C, Colditz GA, Johnson SE: Regulation of progesterone receptor messenger ribonucleic acid and protein levels in MCF-7 cells by estradiol: analysis of estrogen's effect on progesterone receptor synthesis and degradation. Endocrinology 1988, 122:935-944.

Leonard F, Girlanda S, Serio G, Mauri EA, Pyrrome G, Scamperi S, Dalla PP, Barbareschi M: PCNA and Ki67 expression in breast carcinoma: correlations with clinical and biological variables. J Clin Pathol 1992, 45:416-419.

Ursin G, Hovanessian-Larsen L, Parkisy YR, Pike MC, Wu AH: Greatly increased occurrence of breast cancers in areas of mammographically dense tissue. Breast Cancer Res 2005, 7:R605-R608.

Kolonel LN, Henderson BE, Hankin JH, Nomura AMY, Wilkens LR, Pike MC, Stram DO, Monroe KR, Earle ME, Nagamine FS: A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. Am J Epidemiol 2000, 151:346-357.

Maskarinec G, Pagano I, Lurie G, Wilkens LR, Colonel LN: Mammographic Density and Breast Cancer Risk: The Multiethnic Cohorts. Am J Epidemiol 2005, 162:743-752.

Goodman MT, Hernandez BY, Hewitt S, Lynch CF, Cote TR, Frierson HF Jr, Moskaluk CA, Killeen JL, Cozen W, Key CR, et al.: Tissues from population-based cancer registries: a novel approach to increasing research potential. Hum Pathol 2005, 36:812-820.

Anderson WF, Luo S, Chatterjee N, Rosenberg PS, Matsuzone RK, Goodman MT, Hernandez BY, Reichman M, Dollenhall MP, O'Regan RM, et al.: Human epidural growth factor receptor-2 and estrogen receptor expression, a demonstration project using the residual tissue repository of the Surveillance, Epidemiology, and End Results (SEER) program. Breast Cancer Res Treat 2009, 113:189-196.

Shi SR, Key ME, Kalra KL: Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 1991, 39:741-748.

Byng JW, Boyd NF, Fishell E, Jong RA, Yaffe MJ: The quantitative analysis of mammographic densities. Phys Med Biol 1994, 39:1629-1638.

Maskarinec G, Nagata C, Shimizu H, Kashiki Y: Comparison of mammographic densities and their determinants in women from Japan and Hawaii. Int J Cancer 2002, 102:29-33.

Maskarinec G, Pagano I, Chen Z, Nagata C, Gram IT: Ethnic and geographic differences in mammographic density and their association with breast cancer incidence. Breast Cancer Res Treat 2007, 104:47-56.

Simpson ER: Sources of estrogen and their importance. J Steroid Biochem Mol Biol 2003, 86:225-230.

Huang Y, Nagamani M, Anderson KE, Kurowsky A, Haag AM, Grady JJ, Lu LJ: A strong association between body fat mass and protein profiles in nipple aspirate fluid of healthy premenopausal non-lactating women. Breast Cancer Res Treat 2007, 104:57-66.

Yang XR, Charette LA, Garcia-Closas M, Lissowska J, Paal E, Sidaway M, Hewitt SM, Rimm DL, Sherman ME: Construction and validation of tissue microarrays of ductal carcinoma in situ and terminal duct lobular units associated with invasive breast carcinoma. Diagn Mol Pathol 2006, 15:157-161.

Henriksen KL, Rasmussen BB, Lykkefeldt AE, Moller S, Ejlertsen B, Mouridsen HT: Semi-quantitative scoring of potentially predictive markers for endocrine treatment of breast cancer: a comparison between whole sections and tissue microarrays. J Clin Pathol 2007, 60:397-404.

Stueal A, Ma H, Bernstein L, Pike MC, Ursin G: Does breast size modify the association between mammographic density and breast cancer risk? Cancer Epidemiol Biomarkers Prev 2008, 17:621-627.

Bright RA, Morrison AS, Brisson J, Burstein NA, Sadowsky NS, Kopans DB, Meyer JE: Relationship between mammographic and histologic features of breast cancer in women with benign biopsies. Cancer 1988, 61:266-271.

Boyd NF, Jensen HM, Cooke G, Han HL, Lockwood GA, Miller AB: Mammographic densities and the prevalence and incidence of histological types of benign breast disease. Reference Pathologists of the Canadian National Breast Screening Study. Eur J Cancer Prev 2000, 9:15-24.

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