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Original contribution

Digital histologic analysis reveals morphometric patterns of age-related involution in breast epithelium and stroma☆,☆☆

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Summary Complete age-related regression of mammary epithelium, often termed *postmenopausal involution*, is associated with decreased breast cancer risk. However, most studies have qualitatively assessed involution. We quantitatively analyzed epithelium, stroma, and adipose tissue from histologically normal breast tissue of 454 patients in the Normal Breast Study. High-resolution digital images of normal breast hematoxylin and eosin–stained slides were partitioned into epithelium, adipose tissue, and nonfatty stroma. Percentage area and nuclei per unit area (nuclear density) were calculated for each component. Quantitative data were evaluated in association with age using linear regression and cubic spline models. Stromal area decreased ($P = 0.0002$), and adipose tissue area increased ($P < 0.0001$), with an approximate 0.7% change in area for each component, until age 55 years when these area measures reached a steady state. Although epithelial area did not show linear changes with age, epithelial nuclear density decreased linearly beginning in the third decade of life. No significant age-related trends were observed for stromal or adipose nuclear density. Digital image analysis offers a high-throughput method for quantitatively measuring tissue morphometry and for objectively assessing age-related changes in adipose tissue, stroma, and epithelium. Epithelial nuclear density is a quantitative measure of age-related breast involution that begins to decline in the early premenopausal period.

1. Introduction

The human breast undergoes age-related involution, often defined as decreased acinar diameter and decreasing number of acini per terminal ductal lobular unit, with concomitant decreases in mammographic density [1]. Both epithelial involution and decreased mammographic density have been associated with lower breast cancer risk [1-3]. In

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epidemiologic studies of breast cancer risk, mammographic density has been more widely studied than histologic measures of involution. Studies of age-related involution have been impeded by lack of objective and high-throughput histologic measures and by lack of biospecimens for understanding normal breast dynamics with aging [3]. Increasingly, normal breast tissue is available for epidemiologic research [1,4-6], and therefore, reproducible and objective histologic assessment methods are of increasing importance.

We sought to identify morphometric features of mammary gland that vary with age and that could be used as quantitative, objective surrogates for acinar diameter or acini per terminal ductal lobular unit. We applied novel digital imaging algorithms to breast tissue specimens from more than 450 women, including more than 1000 distinct hematoxylin and eosin (H&E) slides. We quantified multiple histologic features and did not restrict our analysis to epithelial features, seeking to also understand how epithelial features relate to other stromal and adipose tissue features. Therefore, our findings identify correlations between morphometric features of stroma and epithelium and illustrate that breast tissue ages as a continuous process beginning in the third decade of life, with epithelium, stroma, and adipose tissue each possessing a unique trajectory of change. Our analysis suggests that the density of nuclei within epithelial regions may function as an objective, high-throughput, and quantitative measure of epithelial involution.

2. Materials and methods

2.1. Study population

The UNC Normal Breast Study (NBS) is a study of breast cancer microenvironment and normal breast tissue conducted at UNC Hospitals in Chapel Hill, NC. Women were eligible for inclusion if they were English speaking, at least 18 years of age, undergoing breast surgery at UNC Hospitals, and consented to donate breast tissue during their surgery. Patients with breast surgeries (mastectomy, lumpectomy, excisional biopsy, reduction mammoplasty, or other cosmetic breast surgery) scheduled between October 2009 and April 2013 were contacted for participation by study personnel during a presurgery appointment with their surgeon. Of 526 patients, 19 (3.6%) declined participation before study consent, and written informed consent was obtained for the remaining 507 patients. A total of 33 patients did not have sufficient available breast tissue at the time of surgery and were ineligible after consent. Thus, the original NBS study population included 399 women with breast cancer and 75 women with benign breast histology for a total of 474 participants.

All participants donated grossly normal-appearing breast tissue (as assessed by pathology assistants at UNC Hospitals) and 2 tubes of blood for lymphocytes, red blood cells, plasma, and serum at the time of surgery. Tissues were snap frozen and/or paraffin embedded, and each patient donated at least 1 tissue sample or multiple specimens, if available. For breast cancer patients, tissue specimens were collected at specified distances from the tumor: less than 1 cm from tumor for patients receiving lumpectomies and less than 1 cm, greater than 1 to 2 cm, greater than 2 to 4 cm, and greater than 4 cm from tumor for patients receiving mastectomies. Patients with benign breast histology donated tissue from 1 or 2 distinct breast sites as available. When possible, bilateral breast tissue was collected.

Participants completed a telephone interview to provide demographic, lifestyle, and breast cancer risk factor exposure data, and medical records abstraction was performed to obtain patients' medical history, mammographic screening, breast cancer–related treatment, tumor pathology, and breast surgery data. Follow-up of medical records is currently ongoing and conducted annually for 10 years after surgery to obtain patient vital status and updated breast cancer recurrence, metastasis, new cancer primaries, and cancer-related treatment data. All study protocols were approved by the UNC School of Medicine's Institutional Review Board.

2.2. Tissue processing and slide preparation

Frozen tissue specimens of approximately 100 mg were cut over dry ice, and sections were collected at both ends of the specimen to construct two 20-μm slides per tissue specimen. The 20-μm section width was selected to maintain tissue integrity from nonfixed tissues and to ensure histologically representative samples, as very few samples were unable to produce viable sections at 20-μm width. The central portion of the tissue specimen was used for nucleic acid extraction as described elsewhere [7]. If frozen specimens were not available (2.0% of patients), paraffin-embedded tissues were used for sectioning and digital annotation. We compared frozen and paraffin-embedded sections from a set of patients (n = 90) and found no significant differences in tissue composition according to sectioning protocol (data not shown). All slides were H&E stained, and histologic slides (1-8 per patient) were scanned into high-resolution digital images using the Aperio ScanScope XT Slide Scanner (Aperio Technologies Vista, CA, United States) in the UNC Translational Pathology Laboratory. The slides were scanned at magnification ×20 (0.5 μm/pixel resolution) using line scan camera technology capturing 1-mm stripes across the entire slide; the stripes are aligned, stored, and accessed as an entire digital slide (US Patent 6,711,283; http://www.archpatent.com/patents/6711283). The image bit depth for the slides was 8 bits. The ScanScope XT uses a linear array scanning technique that generates digital slide images that have no tiling artifacts and that are essentially free from optical aberrations along the scanning axis. The scanned slides had quality factor of more than 90 indicating good focusing. Slides with poor