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Original contribution

Telomere length alterations unique to invasive lobular carcinoma☆,☆☆

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Summary Telomeres are nucleoprotein complexes located at the extreme ends of eukaryotic chromosomes and protect chromosomal ends from degradation and recombination. Dysfunctional telomeres contribute to genomic instability, promote tumorigenesis, and, in breast cancer, have been associated with increased cancer risk and poor prognosis. Short telomere lengths have been previously associated with triple-negative and human epidermal growth factor receptor (Her2)–positive ductal carcinomas. However, these investigations have not specifically assessed invasive lobular carcinomas (ILCs), which accounts for 5% to 15% of all invasive breast cancers. Here, we evaluate telomere lengths within 48 primary ILCs with complete characterization of estrogen receptor (ER), progesterone receptor (PR), and Her2 status, including 32 luminal/Her2− (ER+/PR+/Her2−), 8 luminal/Her2+ (ER+/PR+/Her2+), 3 Her2+ (ER−/PR−/Her2+), and 5 triple-negative (ER−/PR−/Her2−) carcinomas. A telomere-specific fluorescence in situ hybridization assay, which provides single-cell telomere length resolution, was used to evaluate telomere lengths and compare with standard clinicopathological markers. In contrast to breast ductal carcinoma, in which more than 85% of cases display abnormally short telomeres, approximately half (52%) of the ILCs displayed either normal or long telomeres. Short telomere length was associated with older patient age. Interestingly, 3 cases (6%) displayed a unique telomere pattern consisting of 1 or 2 bright telomere spots among the normal telomere signals within each individual cancer cell, a phenotype that has not been previously described. Additional studies are needed to further evaluate the significance of the unique bright telomere spot phenotype and the potential utility of telomere length as a prognostic marker in ILC.

1. Introduction

Invasive lobular carcinoma (ILC) represents 5% to 15% of all breast tumors, thus making it the second most prevalent form of breast carcinoma [1–3]. ILC differs biologically and clinically from invasive ductal carcinoma (IDC). ILC occurs more often in older women, has increased incidence secondary to hormonal replacement therapy, tends to maintain hormone receptor signaling via estrogen receptor (ER) and progesterone receptor (PR), is often associated with

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lobular carcinoma in situ (LCIS), responds poorly to neoadjuvant chemotherapy, and metastasizes to unusual sites including the gynecologic and gastrointestinal tracts [4–8].

Telomeres, composed of repetitive (TTAGGG) DNA bound by a 6-member protein complex termed *shelterin*, protect and stabilize chromosome ends. Telomeres function by masking telomere-induced double-strand DNA break damage signals, thereby prohibiting exonucleolytic degradation and chromosomal fusions. However, telomeric DNA can be progressively lost by several mechanisms, including incomplete replication, whereby telomeres are shortened during each cycle of chromosome replication [9]. In normal cells, moderate telomere loss induces tumor suppressive checkpoint pathways, such as cellular senescence or apoptosis, thus halting cell cycle progression before telomere destabilization [10,11]. In contrast, cancer cells corrupt these checkpoints, leading to critical telomere shortening that, in turn, triggers successive rounds of chromosome breakage-fusion-bridge cycles that drive genetic instability [12,13].

Several studies have shown the presence of short telomeres in breast carcinomas, both at the in situ and invasive stages [14,15], and these short telomeres independently predict poor clinical outcomes [16,17]. We have previously shown that short telomere lengths are associated with human epidermal growth factor receptor (Her2)–positive carcinoma and triple-negative (TNBC; ER−/PR−/Her2−) ductal carcinomas [18]. Recently, Martinez-Delgado and colleagues [19] demonstrated that short telomeres correlated with ER negativity in sporadic breast carcinomas, but not in familial breast carcinoma cases. However, in these prior investigations, the telomere status of ILC cases was not specifically assessed.

Here, using a telomere-specific fluorescence in situ hybridization (FISH) assay that provides single-cell resolution of telomere length while maintaining tissue architecture, we evaluated telomere lengths within 48 primary ILCs with complete characterization of ER, PR, and Her2 status, as well as the associated LCIS component of 22 cases. Telomere lengths were correlated with established clinicopathological features.

2. Materials and methods

2.1. Case selection and tissue microarray construction

This study was approved by the institutional review board of the Johns Hopkins Medical Institutions. Thirty-four cases were evaluated on previously constructed tissue microarrays (TMAs) created from archived, formalin-fixed, paraffin-embedded tissues from cases of primary ER+ ILC [20]. In order to minimize any potential sampling error, each TMA consisted of 99 cores measuring 1.4 mm in diameter, with 5 cores taken per tumor to survey intralesional heterogeneity, and an additional core that contained benign lobules as an internal control. When present, LCIS was also sampled and represented on the TMA. In order to assess other molecular subtypes, an additional 14 cases of primary Her2+ or TNBC ILC were evaluated from whole tissue blocks. In total, 48 cases were evaluated, containing 40 luminal cancers subdivided as 32 luminal/Her2− (ER+/PR+/Her2−; also termed *luminal A* [21]), 8 luminal/Her2+ (ER+/PR+/Her2+; also termed *luminal B* [21]), 3 Her2+ (ER−/PR−/Her2+), and 5 TNBC (ER−/PR−/Her2−). All cases were incident breast carcinomas surgically resected at Johns Hopkins Hospital and were confirmed as lobular phenotype by loss of membranous E-cadherin labeling by immunohistochemistry at the time of initial diagnosis. None of the patients had distant metastasis at the time of diagnosis.

2.2. Immunohistochemistry

ER, PR, Her2, and Ki-67 were performed as part of the routine clinical panel. Briefly, hormone expression for ER and PR was scored as labeling intensity (none, weak, moderate, strong) and percentage nuclear labeling (0-100%). Any labeling greater than 1% was considered positive. Her2 IHC expression was scored (0, 1+, 2+, 3+) using the established American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) scoring criteria at the time of diagnosis, using labeling intensity and proportion complete membranous labeling. A tumor was determined to be Her2+ if the IHC score was 3+ or if the Her2/FISH ratio was greater than 2.0. In this series, all but 1 ER+ cases displayed greater than 60% ER labeling, such that they were unequivocally ER positive, with 1 luminal/Her2+ case demonstrating 20% strong ER labeling. In contrast, all cases classified as ER and PR negative showed 0% nuclear labeling.

2.3. Assessment of telomere length using FISH

Telomere lengths were assessed by fluorescence staining for telomeric DNA as previously described [14,18,22], with the following modifications. Briefly, deparaffinized slides were hydrated through a graded ethanol series and placed in deionized water, followed by deionized water plus 0.1% Tween-20. Slides were steamed for 25 minutes in citrate buffer (Vector Laboratories, Burlingame, CA), allowed to cool at room temperature for 5 minutes, and placed in phosphate-buffered saline (PBS) with Tween (Sigma, Bensalem, PA) for 5 minutes. Slides were thoroughly rinsed with deionized water, followed by 70% ethanol for 5 minutes, 95% ethanol for 5 minutes, and then air-dried. Thirty-five microlieters of a Cy3-labeled telomere-specific peptide nucleic acid (PNA) and Alexa Fluor 488-labeled centromere-specific PNA (0.3 μg/mL of telomere PNA, 0.3 μg/mL of centromere PNA, in 70% formamide, and 10 mmol/L Tris, pH 7.5) was applied to each slide, cover-slipped, and denatured for 5 minutes at 84°C. Slides were hybridized overnight at room temperature in a dark, closed humidified container. The next day, slides were washed twice in PNA wash solution (70% formamide, 10 mmol/L