Whole-Exome Sequencing Analysis of the Progression from Non–Low-Grade Ductal Carcinoma In Situ to Invasive Ductal Carcinoma

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ABSTRACT

Purpose: Ductal carcinoma in situ (DCIS) is a nonobligate precursor of invasive breast cancer. Here, we sought to investigate the level of intralesion genetic heterogeneity in DCIS and the patterns of clonal architecture changes in the progression from DCIS to invasive disease.

Experimental Design: Synchronous DCIS (n = 27) and invasive ductal carcinomas of no special type (IDC-NSTs; n = 26) from 25 patients, and pure DCIS (n = 7) from 7 patients were microdissected separately and subjected to high-depth whole-exome sequencing (n = 56) or massively parallel sequencing targeting ≥410 key cancer-related genes (n = 4). Somatic genetic alterations, mutational signatures, clonal composition, and phylogenetic analyses were performed using validated computational methods.

Results: DCIS revealed genetic alterations similar to those of synchronously diagnosed IDC-NSTs and of non-related IDC-NSTs from The Cancer Genome Atlas (TCGA), whereas pure DCIS lacked PIK3CA mutations. Clonal decomposition and phylogenetic analyses based on somatic mutations and copy number alterations revealed that the mechanisms of progression of DCIS to invasive carcinoma are diverse, and that clonal selection might have constituted the mechanism of progression from DCIS to invasive disease in 28% (7/25) of patients. DCIS displaying a pattern of clonal selection in the progression to invasive cancer harbored higher levels of intralesion genetic heterogeneity than DCIS where no clonal selection was observed.

Conclusions: Intralesion genetic heterogeneity is a common feature in DCIS synchronously diagnosed with IDC-NST. DCIS is a nonobligate precursor of IDC-NST, whose mechanisms of progression to invasive breast cancer are diverse and vary from case to case.

Introduction

Ductal carcinoma in situ (DCIS) constitutes a bona fide nonobligate precursor of invasive breast cancer (1) and, due to advances in imaging techniques, is being detected with increasing frequency (2). DCIS and invasive ductal carcinoma of no special type (IDC-NST) have been found to be genetically similar (3, 4). Indeed, as a group, synchronous DCIS and IDC-NSTs show remarkable resemblance in terms of gene expression profiles and gene copy number alterations (CNA; refs. 4–6). Progression to invasive carcinoma has been estimated to occur in up to 40% of untreated DCIS (4, 7–9). The molecular mechanisms driving progression of DCIS to invasive carcinoma remain contentious (10, 11). Various evolutionary models for progression of DCIS to invasive carcinoma have been proposed, including (i) the independent evolution model, which proposes that DCIS and invasive carcinoma are not genetically related and evolve in parallel (12); (ii) the evolutionary bottleneck model, which posits that a single cell gives rise to different clones, followed by selection of a single clone that traverses the basement membrane invading the surrounding stroma (13); and (iii) the multiclonal invasion model, which suggests that evolution takes place in the DCIS, with ensuing generation of multiple subclones that comigrate and coinvade resulting in the establishment of invasive carcinoma (14). In the latter scenario, one could posit that the ability of invading may have been acquired by DCIS cells early in evolution, prior to the development of intralesion heterogeneity, or that invasion rather is a passive phenomenon from the perspective of the cancer cells or is primarily driven by the microenvironment.

Single-cell and bulk tumor sequencing studies conducted by our group (4, 15) and others (14) have revealed vast intratumor heterogeneity in DCIS and provided direct evidence of different mechanisms resulting in the progression of DCIS to invasive carcinoma, including clonal selection and multiclonal invasion (4, 14, 15). Here, through the study of synchronously diagnosed DCIS and IDC-NST and of DCIS that did not progress to invasive carcinoma, we sought to define the genetic heterogeneity of DCIS, and the repertoire of genetic alterations and clonal architecture of synchronously diagnosed DCIS and IDC-NST to catalog the patterns of clonal architecture changes in the progression from DCIS to invasive breast cancer.
Translational Relevance

We analyzed the genomic landscape of synchronously diagnosed ductal carcinoma in situ (DCIS) and invasive ductal carcinomas of no special type (IDC-NST), and of pure DCIS. Our analyses revealed that DCIS are genetically advanced lesions with marked intratumor genetic heterogeneity and harbor genetic alterations similar to those present in synchronous IDC-NSTS. We have also observed that the molecular mechanisms underpinning progression to invasive carcinoma are diverse and vary from case to case, including clonal selection, which was detected in a minority of cases (28%). Our findings provide further support to the notion that DCIS is a nonobligate precursor of IDC-NST and suggest that progression to invasive disease is a multifaceted process. Hence, the development of molecular predictors of progression of DCIS to invasive carcinoma might require incorporating intratobal genetic heterogeneity and/or the individual mechanisms of progression.

Materials and Methods

Subjects and samples

This study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC), and written informed consent was obtained according to the approved protocol. This study is in compliance with the Declaration of Helsinki. Formalin-fixed paraffin-embedded (FFPE) tissue blocks were retrieved from MSKCC’s pathology archives. Samples were anonymized prior to tissue processing. All cases were reviewed by five pathologists (F. Pareja, R. Bi, F.C. Geyer, M. Vahtdatinia, H.Y. Wenh) for diagnosis confirmation following criteria put forward by the World Health Organization (16). Following pathology review, 27 synchronous DCIS and 26 IDC-NST, including two cases of multifocal-multicentric DCIS, and 7 DCIS not associated with invasion (pure DCIS) were included in this study (Table 1). DCIS and IDC-NSTs were separately microdissected from 10–20 micron-thick histologic sections under a stereomicroscope (Olympus SZ61) to ensure a tumor cell content >80%, as described (4). DNA was extracted from tumor and matched normal tissues, confirmed by pathology review to be devoid of neoplastic cells using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturers’ instructions, as described (17). DNA quantitation and quality control were performed using a Qubit fluorometer (Invitrogen) and a TapeStation system (Agilent), respectively. The DNA samples derived from DCIS, IDC-NST, and normal breast tissue away from DCIS or IDC-NST were subjected to whole-exome sequencing (WES; cases 2, 4–8, 10–13, 18–19, 21–26, 28, 30–35, 38, and 40) or to massively parallel sequencing targeting all coding regions of 410 (case 3) or 468 (case 9) cancer-related genes using the MSK Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay (18). Sequencing results of four of the patients included in this study (i.e., cases 14, 15, 16, and 17) were presented in part in Lee and colleagues, Begg and colleagues, and Weigelt and colleagues (19–21). For these cases, the raw WES sequencing data (i.e., FASTQ files) from DCIS, IDC-NST, and normal breast were retrieved and processed using the same bioinformatics pipeline used for the analysis of the samples sequenced solely for the purpose of this study.

Whole-exome and targeted sequencing analyses

WES and MSK-IMPACT targeted sequencing analyses were performed as described in Weigelt and colleagues (21) and Pareja and colleagues (ref. 22; Supplementary Methods).

Clonal frequencies

To estimate the clonal architecture and composition of the lesions from each patient, mutant allelic fractions from all somatic mutations were adjusted for tumor cell content, ploidy, local copy number, and sequencing errors using PyClone (23), as previously described (refs. 19, 24; Supplementary Methods).

Measures of diversity

To quantitate the intralesional genetic heterogeneity of each sample analyzed, we used the Shannon (25) and Gini-Simpson (26) diversity indices, as previously described (ref. 19; Supplementary Methods).

Comparisons with invasive breast cancers from The Cancer Genome Atlas (TCGA)

The numbers of nonsynonymous mutations, mutational frequencies, and CNAs of DCIS were compared with those of IDC-NSTs from TCGA matched by age (20-year intervals), menopausal status, and ER and HER2 status, at a 1:3 ratio (Supplementary Methods).

Mutational signatures

Mutational signatures were inferred from nonsynonymous and synonymous mutations in samples with at least 40 single-nucleotide variants (SNV) using deconstructSigs (27) based on the set of mutational signatures “signature.cosmic” (28), as previously described (24).

Statistical analysis

Statistical analyses were performed using R v3.1.2. For comparisons between categorical variables, Fisher exact test was used, whereas for continuous variables, the Mann–Whitney U test was used. For comparisons of mutation frequencies, P values were corrected for multiple testing using the Benjamini–Hochberg false discovery rate. All tests were two-sided, and P values < 0.05 were considered statistically significant.

Results

Clinicopathologic characteristics of DCIS and IDC-NSTs

Synchronously diagnosed DCIS (n = 27) and IDC-NSTs (n = 26) from 25 patients and pure DCIS (n = 7) from 7 patients (Table 1) were included in this study and subjected to WES or targeted MSK-IMPACT sequencing (Supplementary Methods; Supplementary Fig. S1). The median sequencing depth of tumor and normal samples subjected to WES was 186× (range, 130×–321×) and 126× (range, 103×–239×), respectively, and of tumor and normal samples subjected to MSK-IMPACT was 596× (range, 525×–720×) and 549× (range, 402×–697×), respectively (Supplementary Table S1). The median age at diagnosis was 50 years (range, 26–76 years) and the median tumor size of the IDC-NSTs was 3.5 cm (range, 0.9–3.1 cm). Forty-one percent (14/34) of DCIS were of intermediate nuclear grade and 59% (20/34) of high nuclear grade, whereas 46% (12/26) and 54% (14/26) of IDC-NSTs were of histologic grades 2 and 3, respectively (Table 1). Fifteen of 27 synchronous DCIS (56%) and 14/26 (54%) IDC-NSTs were ER-positive/HER2-negative, 6/27 synchronous DCIS (22%) and 6/26 (23%) IDC-NSTs were ER-negative/HER2-negative, and 6/27 synchronous DCIS (22%) and 6/26 (23%) IDC-NSTs were...
Table 1. Clinicopathologic characteristics of the DCIS and IDC-NSTs included in this study.

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Note: Cases 14, 15, 16, and 17 were retrieved from our previous studies by Lee et al. (19), Begg et al. (20), and Weigelt et al. (21). Abbreviations: DCIS, ductal carcinoma in situ; IDC-NST, invasive ductal carcinoma of no special type; LIQ, lower inner quadrant; LOQ, lower outer quadrant; N/A, not applicable; UIQ, upper inner quadrant; UOQ, upper outer quadrant.

*IDC-NSTs were graded according to the Nottingham grading system (44), and nuclear grading of DCIS was conducted as per the recommendations of the College of American Pathologists (45).
HER2-positive (Table 1). Notably, all but two DCIS (25/27) displayed a receptor status concordant to that of their synchronous IDC-NST. In contrast, 17/DCIS was ER-positive/HER2-positive while the matched 17/DCD was ER-positive/HER2-negative, and 24/DCD was ER-positive/HER2-negative and the matched 24/DCD was ER-positive/HER2-positive (Table 1). Two multifocal/multicentric DCIS were included in this study (Table 1); in multifocal DCIS case 2, the two DCIS foci (2DCISA and 2DCISB) and the two IDC-NSTs (2IDCA and 2IDCB) mapped to the same breast quadrant, whereas in multicentric DCIS case 5, one DCIS (5DCISA) was located in a breast quadrant distinct from that of the IDC-NST (5IDC) and the remaining DCIS (5DCISA; Table 1). The DCIS (24/DCD) and IDC-NST (24/IDC) of case 24 were located in different breast quadrants, and the synchronous DCIS and IDC-NSTs in all other cases were located in the same breast quadrant (Table 1).

DCIS display a repertoire of somatic genetic alterations similar to that of IDC-NSTs.

WES analyses revealed a median of 37 (range, 16–308) nonsynonymous somatic mutations per synchronous DCIS (n = 25) and a median of 44 (range, 13–230) nonsynonymous somatic mutations per IDC-NST (n = 24), respectively (Supplementary Figs. S2A and S2B; Supplementary Table S2). Notably, the repertoires of somatic mutations detected in synchronous DCIS and their respective IDC-NSTs were markedly concordant (Fig. 1A), and the most frequently mutated cancer genes in the IDC-NSTs (n = 26) largely overlapped with those found in synchronous DCIS (n = 27), including TP53 (54% vs. 52%; P > 0.05), PIK3CA (42% vs. 41%; P > 0.05), and GATA3 (23% vs. 26%; P > 0.05; Fig. 1B). In fact, we did not observe statistically significant differences in the mutational frequency of cancer genes between synchronous DCIS (n = 27) and IDC-NSTs (n = 26) analyzed in this study (P > 0.05, Fisher exact test; Fig. 1B). Of the 12 PIK3CA mutations identified in synchronous DCIS and 12 IDC-NSTs, nine (9/12) affected hotspot residues including H1047R/L (DCIS, n = 9; IDC-NST, n = 8) and E545D (IDC-NST, n = 1; Fig. 1A), and 11/12 in DCIS and 10/12 in IDC-NST were predicted to be clonal (Supplementary Table S2). Most GATA3 mutations in synchronous DCIS (6/7) and IDC-NSTs (6/6) were loss-of-function mutations, including frame-shift (DCIS, n = 4; IDC-NST, n = 5), truncating (DCIS, n = 1), and splice-site mutations (DCIS and IDC-NST, n = 1, each; Fig. 1A; Supplementary Table S2). The majority of TP53 mutations identified in synchronous DCIS (13/14) and IDC-NSTs (12/14) were inactivating, and most of them (DCIS, 12/13; IDC-NST, 12/14) were associated with loss of heterozygosity (LOH) of the wild-type allele (Fig. 1A; Supplementary Table S2).

The majority of synchronous DCIS (63%, 12/19) and IDC-NSTs (58%, 11/19) with sufficient number of mutations for accurate mutational signature inference displayed a dominant aging signature (signature 1 or 5), 21% (4/19) of synchronous DCIS and 26% (5/19) of IDC-NSTs displayed a dominant homologous recombination deficiency (HRD)-related signature (signature 3), and 16% (3/19) of synchronous DCIS and of IDC-NSTs displayed a dominant APOBEC signature (signatures 2 or 13; Supplementary Fig. S2G–S2I). Of the cases with a dominant signature 3 case, 2 case harbored a pathogenic BRCA1 E23Vfs*17 germline mutation associated with LOH of the wild-type allele in all DCIS (2DCISA and 2DCISB) and IDC-NST (2IDCA and 2IDCB) foci, case 19 harbored a BRCA1 R496H germline mutation and LOH in the DCIS and IDC-NST, and both the DCIS and IDC-NST of case 25 harbored a somatic BRCA1 L1154Mfs*4 mutation associated with LOH. Case 23 was heterogeneous and displayed a shift from a dominant aging signature 5 in the DCIS to a dominant HRD-related signature 3 in the matched IDC-NST. A genomic basis for HRD could not be identified in the IDC-NST, however (Supplementary Fig. S2G–S2I).

Copy number analysis demonstrated that synchronous DCIS (n = 27) and IDC-NSTs (n = 26) displayed largely comparable copy number profiles, including recurrent 1q and 16p gains and losses of 5q, 6q, 8p, and chromosomes 13 and 22 (Supplementary Fig. S3A). Recurrent amplifications in the synchronous DCIS (n = 27) affected 17q12 (ERBB2, CDK12, LASP1, and MLIL76), 17q22–q23.3 (HLF, MSI2, CLTC, PPMID, and DDX5), 19q12 (CCNE1), 7p11.2 (EGFR), 8q22.2–q24.2 (MYC, COX6C, UBR5, EXT1), 8p11.22–p11.21 (FGFR1, KA76A, HOOK3, and TCEB1), and 8q21.13 (HEY1; Supplementary Fig. S3A). IDC-NSTs harbored recurrent amplifications of 17q12 (ERBB2, CDK12, LASP1, and MLIL76), 17q21.2 (RARA), 10q22.3 (NUTM2B), 19q12 (CCNE1), 7p11.2 (EGFR), and 11q13.3–13.4 (CCND1; Supplementary Fig. S3A). No statistically significant differences in the frequency of CNAs were observed between the synchronous DCIS (n = 27) and IDC-NSTs (n = 26) from this study (Supplementary Fig. S3B–S3I).

To determine whether the genomic landscape of DCIS differs from that of unrelated IDC-NSTs, we compared the synchronous DCIS from this study with IDC-NSTs from TCGA. The number of nonsynonymous somatic mutations of the synchronous DCIS subjected to WES (n = 25) was comparable with that of IDC-NSTs from TCGA matched for age, menopausal status, and receptor status (1:3 ratio; n = 75; P > 0.05, Mann–Whitney U test; Supplementary Fig. S2B). Similarly, synchronous DCIS from this study (n = 27) harbored a comparable frequency of mutations affecting cancer genes included in MSK-IMPACT than IDC-NSTs from TCGA matched by age, menopausal status, and receptor status (1:3 ratio, n = 81; Fig. 1B).

Comparative analysis of the CNAs in synchronous DCIS from this study (n = 27) and IDC-NSTs from TCGA matched by age, menopausal status, and receptor status (1:3 ratio; n = 81) revealed no statistically significant differences (Supplementary Fig. S3B–S3I). To account for potential differences in histologic grade, given that the DCIS of our cohort were of intermediate or high grade, we compared synchronous ER-positive DCIS from our study (n = 20) to ER-positive luminal B IDC-NSTs from TCGA (n = 60) matched at a 1:3 ratio by age, menopausal status, and HER2 status, and observed no statistically significant differences in terms of mutational frequencies or frequency of CNAs (Supplementary Fig. S3B–S3I). The ER-positive synchronous DCIS from our cohort subjected to WES (n = 18), however, had a lower number of nonsynonymous somatic mutations than the ER-positive luminal B IDC-NSTs from TCGA matched by clinicopathologic characteristics (n = 54; Supplementary Fig. S2C).

We then sought to determine whether the repertoire of genetic alterations in DCIS synchronously identified with invasive carcinoma would differ from that of pure DCIS that did not progress to invasion (median follow-up time of 72 months; range, 22–85 months). Our analyses revealed that despite harboring a comparable number of nonsynonymous mutations (Supplementary Fig. S2D), pure DCIS (n = 7) had a numerically lower frequency of TP53 mutations (14% vs. 52%; P > 0.05) and PIK3CA mutations (0% vs. 41%; P > 0.05) than synchronous DCIS (n = 27; Fig. 1C). No differences in the frequency of CNAs were observed (Supplementary Fig. S3B–S3I). Five out of (83%) pure DCIS with sufficient SNVs for accurate mutational signature inference had a dominant mutational signature 1 (aging), and 1/6 (40DCIS) had a dominant mutational signature 3 (HRD-related; Supplementary Fig. S2G–S2I). 40DCIS harbored a BRCA1 E908* germline mutation. No LOH or somatic mutations affecting the wild-type allele were identified. It is possible that the second hit
Repertoire of nonsynonymous somatic mutations in DCIS, synchronously diagnosed invasive ductal carcinomas of no special type, and pure DCIS, and comparison with invasive carcinomas from The Cancer Genome Atlas breast cancer study.

**A.** Recurrent \((n \geq 2)\) nonsynonymous somatic mutations affecting cancer genes identified in synchronously diagnosed DCIS \((n = 27)\), their corresponding invasive ductal carcinomas of no special type \((IDC-NST, n = 26)\), and pure DCIS \((n = 7)\) by WES or targeted capture massive parallel sequencing using MSK-IMPACT. Cases are shown in columns and genes in rows. Clinicopathologic characteristics are shown on the top. Mutations are color-coded according to the legend.

**B–D.** Comparison of the most frequently mutated cancer genes identified in (B) DCIS synchronously diagnosed with invasive carcinoma \((n = 27)\), their corresponding IDC-NSTs \((n = 26)\), and non-related IDC-NSTs from TCGA matched according to age, menopausal status, and ER/HER2 receptor status \((n = 81)\). C, DCIS synchronously diagnosed with invasive carcinoma \((n = 27)\) and pure DCIS \((n = 7)\). D, Grade 2 DCIS \((n = 14)\) and grade 3 DCIS \((n = 20)\). Two-sided Fisher exact test following multiple testing correction. **:** \(P < 0.001\).

**Figure 1.**

DCIS, ductal carcinoma in situ; ER, estrogen receptor; IDC-NST, invasive ductal carcinoma of no special type; indel, insertion and deletion; SNV, single-nucleotide variant.

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affecting BRCA1 in this case either affected a noncoding region of BRCA1 or was of epigenetic nature.

We then sought to determine whether the repertoire of genetic alterations in DCIS (synchronous or pure) varies according to grade. We observed that grade 3 DCIS (n = 19) subjected to WES had a higher number of nonsynonymous somatic mutations than grade 2 DCIS (n = 13; P < 0.01; Supplementary Fig. S2E). Grade 3 DCIS (n = 20) had a statistically significantly higher frequency of TP53 mutations than grade 2 DCIS (n = 14; 75% vs. 0%; P < 0.001; Fig. 1D). No differences in the frequency of CNAs were observed in DCIS according to grade (Supplementary Fig. S3B–S3I).

Finally, we assessed the repertoire of genetic alterations in DCIS according to ER and HER2 status. We observed that ER-positive/HER2-negative DCIS (n = 19) had a lower number of nonsynonymous somatic mutations than ER-negative/HER2-negative DCIS (n = 6; P = 0.01) and HER2-positive DCIS (n = 7; P < 0.01) subjected to WES (Supplementary Fig. S2F). ER-positive/HER2-negative DCIS (n = 21) harbored a numerically higher frequency of mutations in GATA3 (38%) than ER-negative/HER2-negative DCIS (n = 6; 0%; P > 0.05) and HER2-positive DCIS (n = 7; 14%; P > 0.05). TP53 mutations were numerically more frequent in ER-negative/HER2-negative DCIS (100%) than in ER-positive/HER2-negative DCIS (24%; P > 0.05) and HER2-positive DCIS (57%; P > 0.05; Supplementary Fig. S3K). Besides a higher frequency in ERBB2 gene amplification in HER2-positive DCIS compared with ER-positive/HER2-negative DCIS (P < 0.05), no differences in the frequency of gains/losses and amplifications/homologous deletions in DCIS according to ER/HER2 status were detected (Supplementary Fig. S3B–S3I), likely due to the small sample sizes of each subgroup.

Taken together, our findings suggest that pure DCIS that did not progress to invasive carcinoma are less advanced at the genetic level than DCIS synchronously diagnosed with IDC-NSTs, as they lack mutations in key cancer genes recurrently altered in invasive carcinoma, including PIK3CA. In contrast, DCIS synchronously identified with IDC-NST are genetically advanced lesions and display a similar spectrum of genetic alterations to that of their synchronous invasive carcinomas. Genes significantly mutated in IDC-NSTs, such as PIK3CA, TP53, AKT1, and PTEN (29), were also found to be frequently mutated in synchronous DCIS, indicating roles early in the evolution of breast cancers.

**DCIS and synchronously identified IDC-NSTs are often clonally related**

Given the similarities in the repertoire of somatic mutations of DCIS and synchronously diagnosed IDC-NSTs, we sought to determine whether synchronously identified DCIS and IDC-NSTs of a given patient were clonally related. We computed the clonality index, as previously described (19), which, based on SNVs in WES data, assesses the likelihood of two different lesions to share mutations not expected to have occurred by chance. Most synchronous DCIS subjected to WES (92%; 23/25) were found to be clonally related to their corresponding IDC-NSTs (Fig. 2A) and harbored remarkably similar mutational repertoires and copy number profiles (Fig. 1A; Supplementary Fig. S3B–S3I). The DCIS (2DCIS) and synchronous IDC-NST (2IDC) of case 24 were located in different breast quadrants and were not clonally related (Fig. 2A). Our series included two cases of multifocal DCIS (cases 2 and 5). In case 2, we analyzed two anatomically distinct foci of DCIS (2DCISA and 2DCISB) and two synchronously identified foci of invasive carcinoma (2IDCA and 2IDCB) in the same breast quadrant, all of which were found to be clonally related (Fig. 2A). For multicentric DCIS case 5, we profiled two foci of DCIS arising in different breast quadrants (5DCISA and 5DCISB) along with a focus of IDC-NST (5IDC) identified in the same quadrant as 5DCISA. As expected, our analyses revealed that the DCIS and IDC-NST foci arising in the same quadrant (5DCISA and 5IDC) were clonally related, whereas the DCIS present in a different breast quadrant (5DCISB) did not show any clonal relatedness to the other DCIS focus or to the invasive carcinoma (Fig. 2A). These findings support the notion that DCIS is a nonobligate precursor of IDC-NST and demonstrate that genetically unrelated DCIS and invasive carcinoma can co-occur in the same breast.

**Progression of DCIS to IDC-NSTs follows various evolutionary pathways**

We and others have demonstrated that in situ carcinoma including DCIS and lobular carcinoma in situ (LCIS; refs. 14, 15, 19) can display intratumor genetic heterogeneity. In such cases, virtually all tumor cells harbor founder genetic events (i.e., truncal mutations), whereas subclonal populations carry additional genetic alterations (i.e., branch mutations; ref. 30). Moreover, we have previously observed that genetic mechanisms of progression from DCIS to invasive carcinoma appear to be diverse based on the pattern of CNAs detected in these lesions (15).

To define the changes in clonal architecture in the progression of DCIS to invasive carcinoma, we first jointly inferred the clonal composition of matched DCIS (n = 25) and IDC-NST (n = 24) samples subjected to WES by applying PyClone, a Bayesian clustering model (23) to mutant allele fractions, incorporating tumor cellularity, ploidy, and local copy number obtained from ABSOLUTE (31) and FACETS (32). In addition, we have also performed an independent phylogenetic reconstruction based on CNAs using MEDICC (33).

Our analyses revealed that synchronous DCIS and IDC-NSTs are genetically heterogeneous, and that the mechanisms of progression to invasive carcinoma are diverse. This analysis further confirmed that all but two (92%, 23/25) DCIS synchronously diagnosed with IDC-NST subjected to WES were clonally related to their corresponding invasive carcinoma (Figs. 2A–C, 3, 4; Supplementary Fig. S4A–S4T), corroborating our clonality index findings (Fig. 2A). Clonal decomposition analysis based on PyClone revealed that 7/25 (28%) of DCIS (6DCIS, 7DCIS, 14DCIS, 16DCIS, 22DCIS, and 28DCIS) had minor subclones harboring mutations that became dominant in their corresponding IDC-NST (Fig. 2B–C, Supplementary Fig. S4A–S4E). These clonal shifts might be consistent with a model where progression to invasive disease followed a clonal selection evolutionary pattern. In multifocal DCIS case 2, a subclone of 2DCISA, harboring a KDM5C E185Q mutation, became dominant in a separate DCIS focus (2DCISB), suggesting that 2DCISB stemmed from 2DCISA (Fig. 3). Although the two DCIS and two IDC-NSTs from this patient were clonally related, it was not possible to determine whether the IDC-NSTs stemmed from 2DCISA or 2DCISB. In the remaining nine cases, synchronously identified DCIS and IDC-NST did not display clonal selection (Fig. 4; Supplementary Fig. S4F–S4T).

Cancer genes affected by somatic mutations found to be restricted to the IDC-NST of a given case included GATA3, CHD4, PTEN, RAD51B, SOX2, SPEN, SMO, RB1, CFRB, NCOA1, TP53, NBN, GNAQ, KMT2D, and TET2, among others (Figs. 3 and 4; Supplementary Fig. S4A–S4T). Phylogenetic analyses based on copy number profiles revealed gains of 1q and 16p and losses of 11q, 16q, 6q, 8p, 13, and 22 among truncal CNAs of a given case. Private events restricted to the IDC-NST of a given case included gains of 1q and 16p, losses of 16q and 18q, and...
amplification of 11q13.3–11q13.4 (CCND1), 18q21.33–q22.1 (BCL2), 19p13.11 (JAK), 3q26.32–q26.33 (PIK3CA), 8q23.3–q24.21 (MYC, RAD21), 15q11.1–q26.1 (JDH2, NUTM1), 11p15.5 (HRAS), 1p34.2 (MYCL1), 10q22.3 (NUTM2B), 17q12 (ERBB2, CDK12), and 21q22.2 (ERG) among others (Figs. 2C and 4; Supplementary Fig. S4A–S4T). This analysis also confirmed the observation that 5DCISB and 24DCIS lacked CNAs in common with 5DCISA and 5IDC, and with 24IDC, respectively (Fig. 4; Supplementary Fig. S4Q), providing additional evidence to demonstrate that these DCIS developed independently from their corresponding IDC-NSTs in these patients.

Case 7 (Fig. 2C) harbored truncal PIK3CA (H1047R), TP53 (P278A), and ERBB2 (S310F) hotspot mutations and displayed clonal shifts, consistent with clonal selection in the progression to IDC-NST. In this case, the DCIS component (7DCIS; Fig. 2C) had a hypodiploid genome, with a genomic mass of 1.8 as inferred by FACETS, whereas its synchronous IDC-NST (7IDC) was predicted to have undergone
whole-genome duplication (WGD) and had a genomic mass of 3.3. In addition, 7IDC harbored amplification of 3q26.32–q26.33 that includes PIK3CA, of 8q23.3–q24.21 that encompasses MYC and of 11p15.5 that includes HRAS (Fig. 2C). WGD was also detected in cases 13, 17, 18, 22, 25, and 26 (Supplementary Fig. S4A–S4F). In 3/7 (43%) cases with WGD, both the DCIS and the IDC components were found to harbor the WGD event, whereas in 4/7 (57%) cases, the WGD was restricted to the IDC-NST component. These findings support the notion that WGD may occur not only in the advanced setting but also early in the evolution of a breast cancer.

In multifocal DCIS case 2 arising in a BRCA1 germline carrier (BRCA1 E23Vs*17), despite different absolute number of copies, two foci of ER-negative/HER2-negative DCIS (2DCISA and 2DCISB) and of IDC-NST (2IDCA and 2IDCB) in the same breast quadrant harbored LOH of the wild-type allele of BRCA1 (Fig. 3 and Supplementary Fig. S5). Interestingly, a minor subclone of 2DCISA became dominant in 2DCISB, suggesting that DCIS can give rise to a separate DCIS lesion via clonal selection. Nonetheless, 2IDCA and 2IDCB appear to have undergone subsequent progression with the emergence of subclones restricted to each of the DCIS foci (Fig. 3). 16q loss and GNTAB and EZR mutations were private to 2IDCB, whereas mutations affecting GNAS and SOX2 mutations were private to 2IDCA (Fig. 3). In the multicentric DCIS case 5 (Fig. 4), both our analyses based on somatic mutations and on CNAs revealed that one DCIS (5DCISA) was clonally related to the IDC-NST located in the same quadrant (5IDC), whereas a second focus of DCIS (5DCISB), present in a different quadrant, was not clonally related to either 5DCISA or 5IDC (Figs. 2A and 4). The non-related 5DCISB harbored focal amplification of 17q22–23.2, including PPMD, 19p13.3–q13.41 (MLLT1, CD70), 20q12.2–q13.33 (TSHZ2, SS18L1), and 19q13.43 (ZNF471), whereas the IDC-NST (5IDC) harbored a private 15q11.1–q26.1 amplification (NUTM1, IDH2; Fig. 4).

We next sought to compare the intratumor genetic heterogeneity in DCIS that evolved to invasive carcinoma via clonal selection (n = 7) versus that of DCIS, where clonal selection could not be defined based on WES (n = 18) by computing the Shannon and Gini–Simpson diversity indices (19, 25, 26). Our analyses demonstrated that synchronous DCIS that evolved to IDC-NST following a clonal selection pattern (n = 7) harbored significantly higher intraleision genetic heterogeneity than synchronous DCIS that evolved to IDC-NSTs without evidence of clonal selection (n = 18; Shannon index, P < 0.05; Simpson index, P < 0.05; Mann–Whitney U test) as well as pure DCIS (n = 7; Shannon index, P < 0.05; Simpson index, P < 0.05; Mann–Whitney U test; Fig. 5A). No differences in the intraleision genetic heterogeneity indices of DCIS according to histologic grade or compared with IDC-NST of the same histologic grade, or according to ER/HER2 status, were observed (P > 0.05; Mann–Whitney U test; Fig 5B–C).

Taken together, our findings support the notion that DCIS is a genetically advanced lesion with marked intratumoral heterogeneity and that progression of DCIS to invasive carcinoma varies from case to case, with a minority of samples displaying features consistent with the
dominant clone of the IDC-NST constituting a minor subclone in the respective DCIS. Our findings also indicate that WGD may occur both at the DCIS stage or in the progression from DCIS to invasive carcinoma.

Discussion

Here we provide further evidence in support of the notion that DCIS is a nonobligate precursor of invasive carcinoma. We observed that synchronously diagnosed DCIS and IDC-NSTs were often clonally related. Interestingly, two DCIS analyzed here were found to be genetically independent from IDC-NST diagnosed in a distinct breast quadrant. Although we interrogated only two cases with synchronous lesions located in different breast quadrants, these results might suggest that the geographic relationship between DCIS and IDC-NST may be linked to their clonal relatedness, in contrast to our findings in LCIS, as LCIS and invasive lobular carcinoma were found to be clonally related regardless of their anatomic location (19).

Our findings indicate that DCIS synchronously identified with invasive carcinoma are genetically advanced. Notably, methylation profiling of DCIS and adjacent invasive carcinoma supports the notion that, also epigenetically, DCIS is an advanced lesion (34, 35). It is possible that high-grade DCIS might arise de novo. Nonetheless, it has been recently shown that atypical ductal hyperplasia (ADH) might be clonally related to synchronously identified high-grade DCIS and invasive carcinoma, suggesting the possibility that ADH might be a precursor lesion not only in the low-grade but also in the high-grade breast cancer evolutionary pathways (36).

Notably, in agreement with Moelans and colleagues (37), we did not identify significant differences in the frequency of CNAs between synchronous DCIS compared with IDC-NST and to pure DCIS. Furthermore, amplification of CCND1 and MYC, found to display an increased amplitude in invasive carcinoma compared with DCIS (38), was restricted to the invasive component of a given case in our series. Previously reported higher frequencies of 5q31.1–5q33.3, 6q25.3–6q26, and 13q23.3–13q33.1 losses and 11p12 gains in DCIS compared with their synchronously identified invasive carcinoma (39), and a higher frequency of 1q, 8q, and 11q gains in synchronous compared with pure DCIS (40, 41) could not be confirmed in our study. Such discrepancies might stem from the small sample size of DCIS in our cohort and the fact that low-grade DCIS samples were not included in our study. Consistent with Sakr and colleagues (42), all pure DCIS in our study lacked PIK3CA mutations.
mutations. These findings contrast the observations by Lin and colleagues (41), who reported an enrichment in PIK3CA kinase domain mutations in pure compared with synchronous DCIS. Larger studies of DCIS/invasive carcinomas carefully stratified by grade and ER and HER2 status are warranted.

By performing clonal decomposition based on somatic mutations and a phylogenetic reconstruction based on copy number profiles of DCIS and synchronously diagnosed IDC-NSTs, we observed that both DCIS and IDC-NSTs are genetically heterogeneous and that progression to invasive carcinoma may follow different evolutionary pathways. In 7 of 25 cases (28%), the clonal decomposition and/or CNA analyses supported the hypothesis that the invasive carcinoma stemmed from a minor subclone of the synchronously diagnosed DCIS, following a clonal selection evolutionary pattern. PTEN and GATA3, TP53, and KMT2D were among the cancer genes affected by nonsynonymous somatic mutations restricted to the invasive component of individual cases. In addition, we observed amplification of cancer genes restricted to the IDC-NST component of a given case, including CCND1, PIK3CA, IDH2, MYC, MYCL1, ERBB2, and HRAS, suggesting that these may play a role in the progression from DCIS to invasive carcinoma. In the majority of cases (72%), however, our analyses did not show any evidence of clonal selection during progression to invasive carcinoma. It is plausible that in these cases, genetic alterations conferring ability to invade were acquired early in their development, followed by the acquisition of intralesion genetic heterogeneity and multiclonal invasion. Alternatively, invasion in these cases might have been driven by changes in the microenvironment or merely constituted a passive phenomenon.

WGD has been reported in up to 30% of advanced cancers (43). Here, we demonstrate that this phenomenon may be present even at the DCIS stage. In three cases analyzed here, WGD was a truncal event, suggesting that it took place relatively early in tumor evolution, and in four cases, WGD was restricted to the IDC-NST, illustrating the profound differences that can be observed between the modal populations of cancer cells from a DCIS and its synchronously diagnosed IDC-NST. One could posit that WGD occurring early in tumor development could result in increased levels of genome instability and increased intratumor heterogeneity, facilitating the accumulation of additional genetic alterations, whereas in other contexts, WGD may occur relatively late and was either present in a minor subclone of the DCIS that came to become the dominant clone of the IDC-NST or one of the potential mechanisms underpinning progression to invasive breast cancer.

Figure 5.
Intratumor genetic heterogeneity of DCIS. Boxplots depicting the Shannon diversity index (top) and the Gini–Simpson diversity index (bottom) (A) in DCIS synchronously diagnosed with invasive carcinoma displaying a clonal selection evolutionary pattern (n = 7), synchronous DCIS lacking evidence of clonal selection (n = 18), and in pure DCIS (n = 7); (B) in grade 2 (n = 15) and grade 3 (n = 19) DCIS, and grade 2 (n = 10) and grade 3 (n = 14) IDC-NST; (C) in ER-positive/HER2-negative DCIS (n = 19), ER-negative/HER2-negative DCIS (n = 6), and HER2-positive DCIS (n = 7) subjected to WES. The median value of the Shannon diversity index and the Gini–Simpson diversity index, and the 75th and 25th percentiles are displayed at the top and bottom of the boxes, respectively. Each dot corresponds to the Shannon or Gini–Simpson diversity index of one case. Mann–Whitney U test two-sided P values. ‘*’ P < 0.05.
Our study has important limitations. First, our study was restricted to intermediate- and high-grade DCIS, and did not include low-grade DCIS. Second, we used tumor bulk sequencing and state-of-the-art computational approaches to infer the clonal architecture of each sample/case and their phylogeny. Although these were bioinformatics inferences, they were consistent with the results of single-cell sequencing studies carried out by our group (15) and others (14). Finally, we have focused on somatic genetic alterations affecting protein coding genes; further studies investigating potential mechanisms of progression from DCIS to invasive disease based on somatic genetic alterations affecting non-coding regions of the genome are warranted.

Despite these limitations, our findings corroborate the notion that DCIS is a neoplastic nonobligate precursor of invasive carcinoma, with a repertoire of somatic genetic alterations similar to those of IDC-NSTs. Most importantly, our results highlight the complexity of evolutionary patterns driving progression from DCIS to invasive carcinoma and suggest they may vary from case to case. In fact, in a minority of patients (7 of 25; 28%), evidence of clonal selection in the evolutionary patterns driving progression from DCIS to invasive disease was documented, and in these cases, higher levels of intratissue genetic heterogeneity were observed. These observations support the contention that biomarkers to predict the progression from DCIS to invasive disease ought to consider the information provided by intratissue genetic heterogeneity and the potential mechanisms of disease progression.

Data availability

The WES and targeted sequencing data that support the findings of this study will be available for visualization and download in cBioPortal for Cancer Genomics (http://www.cbioportal.org) upon publication of the manuscript.

Disclosure of Potential Conflicts of Interest

J.S. Reis-Filho is an employee/paid consultant for Goldman Sachs, Paige.AI, Voltion Rx, InVivo, Roche Tissue Diagnostics, Genentech, Novartis, and Roche. No potential conflicts of interest were disclosed by the other authors.

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References


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