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Review

Progression from ductal carcinoma *in situ* to invasive breast cancer: Revisited

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ABSTRACT

Ductal carcinoma *in situ* (DCIS) is an intraductal neoplastic proliferation of epithelial cells that is separated from the breast stroma by an intact layer of basement membrane and myoepithelial cells. DCIS is a non-obligate precursor of invasive breast cancer, and up to 40% of these lesions progress to invasive disease if untreated. Currently, it is not possible to predict accurately which DCIS would be more likely to progress to invasive breast cancer as neither the significant drivers of the invasive transition have been identified, nor has the clinical utility of tests predicting the likelihood of progression been demonstrated. Although molecular studies have shown that qualitatively, synchronous DCIS and invasive breast cancers are remarkably similar, there is burgeoning evidence to demonstrate that intra-tumor genetic heterogeneity is observed in a subset of DCIS, and that the process of progression to invasive disease may constitute an 'evolutionary bottleneck', resulting in the selection of subsets of tumor cells with specific genetic and/or epigenetic aberrations. Here we review the clinical challenge posed by DCIS, the contribution of the microenvironment and genetic aberrations to the progression from *in situ* to invasive breast cancer, the emerging evidence of the impact of intra-tumor genetic heterogeneity on this process, and strategies to combat this heterogeneity.

1. Introduction

Ductal carcinoma *in situ* (DCIS) is defined as a premalignant proliferation of neoplastic epithelial cells contained within the lumen of mammary ducts. DCIS are lined by a layer of semi-continuous myoepithelial cells and surrounded by an intact basement membrane (Lopez-Garcia et al., 2010). For several decades it has been accepted that DCIS constitutes a non-obligate precursor of invasive ductal carcinoma. In 1973, Wellings and Jensen proposed that non-malignant ductal lesions are precursors of invasive breast cancer (IBC) based on the evidence of gradual histological continuity observed in

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normal and abnormal breast tissues (Wellings and Jensen, 1973). This theory is supported by a plethora of succeeding work aiming to characterize DCIS and IBC at a molecular level, which have revealed their genetic similarity and likely common origin (Buerger et al., 1999a, 1999b; Burkhardt et al., 2010; O'Connell et al., 1998).

Clinical observational studies have further corroborated the hypothesis that DCIS is a precursor of IBC. Perhaps the most persuasive evidence to suggest that DCIS and IBC are progressive stages of an evolutionary continuum is that they affect the same anatomical site. Longitudinal studies of patients with DCIS managed with biopsy only revealed that 20–50% of this population later developed IBC in the same quadrant of the same breast as the original DCIS (Page et al., 1995, 1982; Sanders et al., 2005). DCIS is found adjacent to invasive disease in the vast majority of IBCs at the time of diagnosis (Evans et al., 1997; Fisher et al., 1975), where it was thought to be the precursor lesion, however the coexistence of DCIS with IBC varies according to the subtype of breast cancer (Abdel-Fatah et al., 2007). DCIS can be classified into similar molecular subtypes as IBC based primarily on the expression patterns of ER, PR, HER2, EGFR and cytokeratin 5/6 (Bryan et al., 2006; Clark et al., 2011; Livasy et al., 2007; Muggerud et al., 2010), and associated *in situ* and invasive components often, but not always (see below), exhibit a similar immunophenotype (Steinman et al., 2007; Tamimi et al., 2008). Also, nuclear grade is generally concordant between *in situ* and invasive components of invasive carcinomas, which have comparable nuclear morphology (Giardina et al., 2003) and DNA ploidy (Ottesen, 2003).

DCIS has become a formidable clinical challenge due to its increasing incidence. In fact, 54,944 diagnoses of DCIS are expected in 2013 according to the American Cancer Society, up from 45,900 cases in 2010, and now DCIS accounts for approximately 20% of all breast cancers (American Cancer Society, 2013). This rapid increase in the incidence of DCIS parallels the introduction of mammography screening, as the majority of DCIS lesions are detected upon stereostatic biopsy of mammographic microcalcifications (Virnig et al., 2010). Although there have been numerous efforts to develop clinical or molecular tests (Solin et al., 2013) to predict which patients are likely to develop invasive disease following a diagnosis of DCIS, there is currently no test with demonstrated clinical utility to identify this population. Hence, the vast majority of patients are still subjected to surgical treatment followed by radiation and/or prophylactic systemic therapies (e.g. tamoxifen). Further, despite extensive efforts to unravel the biological underpinnings of the phenomenon of progression from *in situ* to invasive disease and to develop biomarkers predictive of the likelihood of progression to IBC, several biological aspects of the transition from *in situ* to invasive disease have yet to be elucidated and there are still no histopathological or molecular markers to predict accurately the progression from DCIS to IBC.

Here we discuss the biological processes that are likely to play a role in the progression from *in situ* to invasive disease, the challenges posed by intra-tumor genetic heterogeneity and potential strategies to develop biomarkers to define the subsets of DCIS that are likely to progress to IBC.

2. The role of the microenvironment in progression from DCIS to IBC

The theories of progression from DCIS to IBC fall broadly into two categories: those, which consider invasiveness as an acquired behavior that stems from genetic aberrations occurring in the neoplastic cells, and those, which suggest it is independent of additional genetic changes within the lesion. The most popular 'non-genetic' theory is that the microenvironment or tumor stroma actively drives tumor progression. Several studies have examined the pro-invasive influence of the extracellular matrix (ECM) and non-tumoral cells on DCIS. For example, Lyons and colleagues (Lyons et al., 2011) suggested that mammary gland involution post-pregnancy is a driving force of tumor progression as a result of re-modeling of the ECM. Using a murine model of postpartum involution, the authors observed that MCF10ADCIS.com cells subjected to the involution microenvironment formed large tumors with increased intra-tumor collagen deposition and expression of cyclooxygenase-2 (COX-2). In this model, both fibrillar collagen and COX-2 were required for the acquisition of an invasive phenotype, which could be at least in part blocked by non-steroidal anti-inflammatory drugs (Lyons et al., 2011). Hu and colleagues also found that stromal fibroblasts increased invasion in a xenograft model of DCIS in part by increasing expression of COX-2 in tumor epithelial cells (Hu et al., 2009). Other studies have linked elevated stromal cell expression of Lysyl oxidases (LOXs), a family of ECM modifying enzymes, to invasion and metastasis (Barker et al., 2011; Levental et al., 2009).

Gene expression profiling has revealed that substantial changes occur during progression from DCIS to IBC in various cell types of the tumor microenvironment, including fibroblasts, myoepithelial cells and leukocytes (Allinen et al., 2004; Ma et al., 2009; Vargas et al., 2012). The underlying causes of these differences in gene expression are still unclear. As expected, no clonal genetic aberrations were detected in the myoepithelial cells and fibroblasts surrounding DCIS or IBC (Allinen et al., 2004; Qiu et al., 2008). Consequently, it has been suggested that epigenetic alterations in the stroma may be involved in the progression from *in situ* to IBC (Hu et al., 2005).

Histologically, the major difference between DCIS and IBC is that the former retain an outer layer of myoepithelial cells and an intact basement membrane. A commonly touted theory of progression is that the normal myoepithelium acts as a 'gatekeeper', exerting tumor suppressive effects on the *in situ* lesion, and that it is the loss of this suppressive environment that unleashes the progression to invasive disease (Barsky and Karlin, 2005; Gudjonsson et al., 2005; Place et al., 2011; Polyak and Hu, 2005). As well as forming a physical barrier to invasion, myoepithelial cells also secrete various ECM components and protease inhibitors, such as Maspin, proposed to inhibit the invasive capacity of DCIS in a paracrine manner (Barsky and Karlin, 2005, 2006; Sternlicht et al., 1997). Hu and colleagues provided support for this theory upon finding that co-transplantation of normal myoepithelial cells prevented occurrence of invasive disease in a xenograft model of human DCIS, whereas

fibroblasts enhanced tumor growth and invasion (Hu et al., 2008). Such mechanisms of progression may partially explain the lack of differences detected between DCIS and IBC at the genomic level.

Epigenetic alterations, namely inheritable changes in gene expression that do not involve changes in DNA sequences, within neoplastic cells could also account for the current lack of significant differences detected between DCIS and IBC at DNA sequence level. DNA methylation commonly increases from normal breast epithelium to DCIS, however the majority of studies have found similar levels of promoter hypermethylation in DCIS and IBC (Moelans et al., 2011b; Park et al., 2011; Verschuur-Maes et al., 2012). This suggests that alterations in DNA methylation are early events in breast carcinogenesis rather than key factors in the transition to invasive disease. Consistent with this observation, an increase in the number of methylated genes has been documented in columnar cell lesions, the earliest morphologically identifiable precursor of breast cancer, as compared to normal breast epithelium, however no differences between the number of methylated genes between columnar cell lesions, DCIS and IBC were observed (Verschuur-Maes et al., 2012). Given the recent data from the Encyclopedia of DNA Elements (ENCODE)(Encode Project Consortium, 2012) suggesting that methylation is likely to be a consequence rather than a cause of gene silencing (Sproul et al., 2011), alternative epigenetic events may be even more important in the progression from *in situ* to IBC than methylation. In fact, changes in chromatin states could play a role in progression as global changes in histone modifications that mark heterochromatin and euchromatin have been implicated in epithelial-to-mesenchymal transition (EMT)(McDonald et al., 2011), which is reported to be associated with progression to IBC from DCIS (Knudsen et al., 2012). Therefore, studies investigating the role of epigenetic alterations in the progression from *in situ* to IBC are warranted.

3. Genetic alterations in the progression to invasive disease

Numerous lines of evidence demonstrate that DCIS is a non-obligate precursor of IBC and that DCIS harbors genetic aberrations similar to those found in synchronous and metachronous IBC developing in the same quadrant. Importantly, however, robust transcriptomic or genomic signatures to distinguish DCIS from IBC have proven elusive. Several gene expression profiling analyses of premalignant, preinvasive and IBCs to determine whether gene expression patterns could be used for diagnostic or prognostic purposes have been performed. These studies have shown that at the transcriptomic level, preinvasive lesions and invasive breast cancer of the same histological grade display remarkably similar gene expression patterns and that it is not possible to identify gene signatures that discern between the pathological stages of DCIS and IBC robustly (Ma et al., 2003; Vincent-Salomon et al., 2008). Overall, analyses of chromosomal aberrations by array-based comparative genomic hybridization (aCGH) have been no more fruitful in clearly discriminating DCIS from IBC (Gao et al., 2009; Liao et al., 2012; Yao et al., 2006). DCIS and invasive components from the same patients are frequently found to be closely related not only on the basis of their gene expression but also gene copy number aberrations (Johnson et al., 2012; Lee et al., 2012; Liao et al., 2012; Moelans et al., 2011a; Porter et al., 2003). Although these findings lend further support to the notion that DCIS is a precursor of IBC, they have also been interpreted as evidence to suggest that progression from *in situ* to invasive disease is not necessarily driven by specific genetic aberrations in DCIS cells.

Although DCIS and IBC appear genetically similar, some qualitative differences have been found between matched DCIS and IBC (Table 1). Studies on the prevalence of HER2 overexpression and *HER2* gene amplification provide examples where clear genomic differences are observed between

Table 1 – Common gene amplifications found to be distinct between DCIS and synchronous adjacent IBC.

Gene	Study	Number of paired DCIS and IBC cases	% cases restricted to DCIS	% cases restricted to IBC	% cases present in both components	% of cases absent in both components
MYC	Jang et al. (2012)	203	1	3	8.9	87.1
	Robanus-Maandag	12	0	1	NA	NA
	et al. (2003)	14	0	5	4	5
	Burkhardt et al. (2010)	135 (71 analyzable)	5.6	0	5.6	88.7
HER2	Jang et al. (2012)	203	1	0	22.8	76.2
	Burkhardt et al. (2010)	135 (74 analyzable)	5.4	0	16.2	78.4
	Park et al. (2006)	270	1.5	0	28.5	70
FGFR1	Jang et al. (2012)	203	0	3.1	10.3	86.6
CCND1	Jang et al. (2012)	203	1.5	0.5	15.9	82.1
	Burkhardt et al. (2010)	135 (73 analyzable)	0	2.7	11.0	86.3

Latta et al. (Latta et al., 2002) reported complete concordance in *HER2* amplification between the *in situ* and invasive components in all but one case analyzed by fluorescence *in situ* hybridization (FISH); in this case *HER2* amplification was found to be present in the *in situ* but absent in the invasive component (total number of paired DCIS and IBC cases analyzed by FISH not available). DCIS, ductal carcinoma *in situ*; IBC, invasive breast cancer; NA, not available.

adjacent invasive tumors and *in situ* lesions or within *in situ* lesions (Figure 1). In a small percentage of HER2-positive tumors amplification of the *HER2* locus was found to be present in the DCIS but not the associated IBC (Burkhardt et al., 2010; Latta et al., 2002; Park et al., 2006), and some cases of DCIS showed overt heterogeneity of HER2 overexpression within the DCIS component (Figure 1). These data indicate that either *HER2* amplification may have been lost during progression to invasive disease or that the invasive component arose from a clone in the DCIS that did not harbor *HER2* amplification in the first place. Recent studies have found evidence for convergent phenotypic evolution in tumor progression and metastasis (Gerlinger et al., 2012), and it is plausible that progression from DCIS to IBC also constitutes a convergent phenotype (i.e. it may be caused by numerous genetic and/or epigenetic mechanisms, Figure 2A). In fact, the hypothesis that progression from *in situ* to IBC constitutes a convergent phenotype provides a potential explanation for the negative results in the genomic and transcriptomic comparisons between DCIS and IBC.

The majority of studies comparing DCIS and IBC have not focused solely on synchronously occurring ipsilateral samples (Liao et al., 2012; Robanus-Maandag et al., 2003). Such studies have suggested that there might be copy number aberrations associated with progression from DCIS to IBC, including *MYC* (Robanus-Maandag et al., 2003), *FGFR1* (Jang et al., 2012), and *CCND1* (Burkhardt et al., 2010) gene amplification, which were shown to be more frequently observed in IBC than in DCIS (Table 1). It should be noted, however, that these genetic aberrations do not appear to be either necessary or sufficient for the acquisition of an invasive phenotype (Burkhardt et al., 2010; Jang et al., 2012; Liao et al., 2012; Park et al., 2006; Robanus-Maandag et al., 2003). In fact, in some cases of paired DCIS and IBCs, when amplifications of the *MYC* and *CCND1* loci have been found, they were reported to be restricted to the DCIS component, to the invasive component or to be concurrently present in both (Burkhardt et al., 2010; Jang et al., 2012; Robanus-Maandag et al., 2003) (Table 1). The approaches employed so far, looking for common transcriptomic and/or genomic differences between matched synchronous DCIS and IBC, would fail to identify significant differences if progression to invasive disease is a convergent phenotype without a given mechanism to account for the majority of cases. Consistent with this hypothesis, recent studies based on pairwise comparisons between DCIS and IBC have revealed the existence of significant genetic differences, however these differences are distinct from patient to patient (Hernandez et al., 2012; Heselmeyer-Haddad et al., 2012). One could posit that drivers of progression may be masked when DCIS and IBC are compared as groups and not matched entities if distinct drivers of progression are specific to individual cases or a small number of patients (Hernandez et al., 2012). It is also plausible that these distinct genetic and/or epigenetic aberrations may target similar or complementary pathways that would result in similar biological outputs (i.e. progression from *in situ* to invasive disease)(Yap et al., 2012). In addition, limitations in the types of the genetic aberrations detected by the techniques used (e.g. somatic point mutations and small insertions and deletions, somatic rearrangements and copy number aberrations) may have resulted in the apparent lack of genetic differences between DCIS and synchronous adjacent IDC.

4. Genetic heterogeneity and clonal evolution in DCIS and IBC

Recent in-depth genetic analyses of synchronous ipsilateral DCIS and IBC have attempted to determine both intra-tumor genetic heterogeneity in both components and genetic differences acquired during progression to IBC (Hernandez et al., 2012; Heselmeyer-Haddad et al., 2012). Remarkably, both studies found not only extensive genetic heterogeneity, in the form of the percentage of neoplastic cells harboring amplification of specific loci in DCIS, but also presented some evidence supporting the idea of clonal selection during the progression toward invasive disease, suggesting that transition from DCIS to IBC may constitute an evolutionary bottleneck (Turner and Reis-Filho, 2012; Yap et al., 2012) (Figure 2B).

Our group performed aCGH, fluorescence *in situ* hybridization (FISH) and Sequenom analysis on 13 synchronous adjacent DCIS and IBC sets and found that matched DCIS and IBC had strikingly similar genomic profiles (Hernandez et al., 2012), in agreement with the results of previous studies.

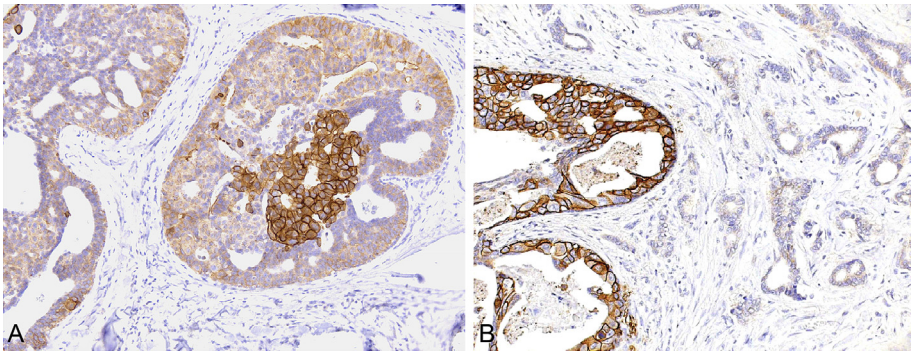

Figure 1 – Heterogeneous expression of HER2 in breast cancer. (A) Heterogeneous expression of HER2 in neoplastic cells of a ductal carcinoma *in situ*; note that only a subpopulation of the cancer cells in one duct display strong, membranous expression of HER2. (B) Representative micrograph illustrating a ductal carcinoma *in situ* composed of cells displaying HER2 membranous expression associated with a HER2-negative invasive ductal carcinoma.

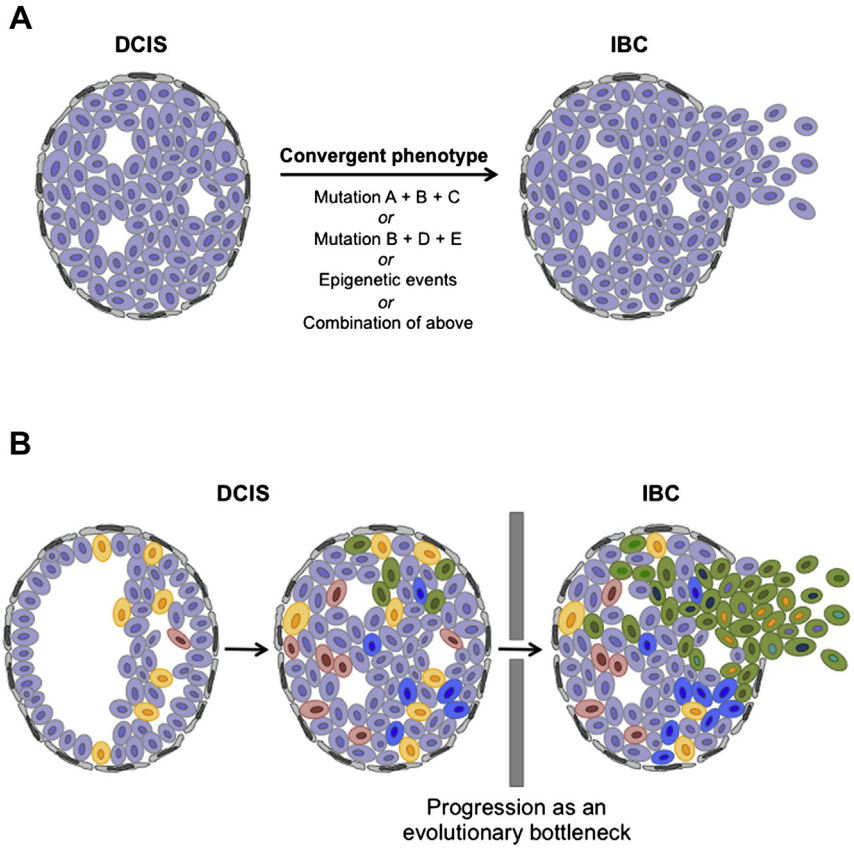

Figure 2 – Hypothetical models of progression from *in situ* to invasive breast cancer. (A) Progression from DCIS to IBC as a convergent phenotype, where several combinations of somatic genetic and/or epigenetic aberrations result in the acquisition of the biological properties required for cancer cells to progress from *in situ* to invasive disease (i.e. the genetic/epigenetic aberrations selected for are distinct between patients but all result in the progression to invasive disease). (B) Progression from DCIS to IBC as an evolutionary bottleneck. As DCIS develops, cells accumulate somatic mutations and copy number aberrations (depicted by color) to generate a heterogeneous lesion with distinct subclones harboring private mutations in addition to the founder genetic aberrations present in all neoplastic cells. Only subclones harboring a specific repertoire of genetic aberrations are selected and pass through the evolutionary bottleneck of progression to IBC. DCIS: ductal carcinoma *in situ*; IBC: invasive breast cancer.

Importantly, however, not only intra-lesion genetic heterogeneity but also differences in the prevalence of specific amplifications and mutations between the DCIS and invasive samples were detected, consistent with a model where DCIS is composed of a mosaic of tumor cells that in addition to the founder genetic aberrations harbor private mutations, and that clonal selection does take place in the progression from *in situ* to invasive disease. For example, three of 13 microdissected cases harbored *PIK3CA* mutations which were restricted to the DCIS component in two cases, and in a third case the frequency of the *PIK3CA* mutant allele decreased from 49% to 25% in the IBC component (Hernandez et al., 2012).

Heselmeyer-Haddad and colleagues used FISH to probe eight genomic loci frequently lost or gained in breast cancer in single cells microdissected from matched DCIS and IBC samples (Heselmeyer-Haddad et al., 2012). Through the analysis of patterns of gains and losses in single cells it was also concluded that DCIS exhibit a high level of intra-tumor genetic heterogeneity and that matched DCIS and IBC have similar but not identical patterns of genomic imbalances. Differences between matched pairs of DCIS and IBC were detected; in four cases a switch from DCIS to IBC in the modal clone was detected and associated with a gain of *MYC* in more than 50% of the cells they analyzed. This suggests that specific genetic aberrations are selected for during progression to IBC, but that these changes may vary from patient to patient, consistent with the hypothesis that progression from *in situ* to IBC may constitute a convergent phenotype.

These studies highlight the importance of i) comparing synchronous ipsilateral samples of DCIS and IBC, as the genetic differences observed between pairs of synchronous DCIS and IBC would probably not have been detected in unmatched samples, and ii) using methods that provide quantitative information on genotype and copy number status, as most differences would not have been detected if single cells had not been analyzed or if semi-quantitative methods had not been employed. Taken together, these observations provide a tantalizing glimpse into the progression of DCIS to IBC, and support the theory that this process is a result of

selection of subpopulations of neoplastic cells. Although previous work support the idea that intra-tumor heterogeneity exists in DCIS (Aubele et al., 2000, 1999; Fujii et al., 1996; Hu et al., 2008), these recent studies provide strong circumstantial evidence to suggest that progression from DCIS to IBC may occur, at least in some samples, by Darwinian selection.

5. Design of future studies of the progression of DCIS to IBC

To obtain conclusive answers as to whether progression from DCIS to IBC is a result of intra-lesional genetic heterogeneity and clonal selection, it will be necessary to enter the rapidly expanding field of massively parallel sequencing. Over the past few years the cost and speed of DNA sequencing has plummeted facilitating an increase in the depth of sequencing (Mwenifumbo and Marra, 2013). The unprecedented level of genetic information provided by massively parallel sequencing has allowed for a progressively more detailed picture of inter- and intra-tumor genetic heterogeneity and the process of tumor evolution. On an intra-individual scale studies have found the existence of genetic differences between primary breast cancers and metastases that support the hypothesis of clonal evolution (Ding et al., 2010; Shah et al., 2009). Shared genomic aberrations exist between primary breast tumors and their metastatic deposits suggesting common ancestry, however the prevalence of specific mutations in the cells within each lesion have been shown to differ. This indicates that in the tumors studied, a subpopulation of cells from the primary tumor gave rise to the metastasis. In addition, aberrations have been found that were specific to the metastasis potentially indicating continued evolution (Ding et al., 2010; Shah et al., 2009). On an intra-tumor level multi-region sequencing of tumors has confirmed the existence of intra-tumor genetic heterogeneity (Gerlinger et al., 2012; Navin et al., 2011, 2010), which is a pre-requisite of clonal evolution.

Increased depth of sequencing provides the ability to detect point mutations and copy number alterations present in minute fractions of a tumor population (the exact fraction depends on numerous factors, including the number of sequencing reads, cellularity, allelic frequency, tumor ploidy, and patterns of gene copy number aberrations). This information when integrated by computational algorithms can be used to infer the prevalence of subclonal populations within a tumor, or between tumor sites to address cancer genome evolution (Nik-Zainal et al., 2012; Shah et al., 2012). These methodologies have been used to elucidate the subclonal populations present in breast cancers and the likely order of occurrence of mutations in their evolution, dramatically increasing our knowledge of the mutational landscape of these cancers (Nik-Zainal et al., 2012; Shah et al., 2012). Bioinformatic tools to analyze massively parallel sequencing data are continuously improving to allow more detailed analysis from sequencing from bulk tumor populations, with more sensitive methods to identify somatic point mutations (Cibulskis et al., 2013), and to determine tumor purity and cell ploidy (Carter et al., 2012). These methods were recently employed to elucidate the frequency of subclonal driver mutations present in chronic lymphocytic leukemia, and their effect on clinical outcome from whole exome sequencing data (Landau et al., 2013). These analyses have not only revealed that the presence of subclonal driver mutations is associated with poorer outcome, but also that chemotherapy acted as an evolutionary bottleneck, leading to selection and ulterior expansion of specific driving subclones (Landau et al., 2013). Based on the assumption that DCIS is the precursor lesion of IBC, one could exact a similar approach with matched DCIS and IBC, treating them as longitudinal time points. High-depth whole genome or whole exome sequencing complemented by independent analyses of copy number and allelic frequencies would comprehensively address the level of genetic heterogeneity of DCIS and IBC and whether progression to invasive disease truly constitutes an evolutionary bottleneck.

Such analysis on bulk tumor material could be enhanced by complementary evaluations at the ultimate resolution of genetic heterogeneity, the single cell. To date, one can potentially determine the presence of genomic aberrations in subclonal populations of a tumor by analyzing copy number variations at a single cell level (Navin et al., 2011). These methods, combining subclonal analysis by copy number with the lineage analysis based on mutation frequency from deep sequencing of bulk tissue, have the potential to conclusively answer whether progression from *in situ* to IBC is results from the selection of a subpopulation of DCIS cells harboring a specific genotype (Figure 3).

Following on from DNA sequencing, functional annotation of these findings could then be used to determine whether progression to IBC constitutes a convergent phenotype and whether specific signaling pathways or networks are involved. Additionally, *in vitro* and *in vivo* models of DCIS would be necessary to confirm the roles of potential drivers of progression, and to determine whether drivers may provide novel therapeutic targets or prognostic markers. Although such *in vitro* and *in vivo* models that accurately represent the biology of DCIS are scarce, a handful of cell lines and xenograft models have been used with some success to recapitulate a DCIS-like phenotype. For example, the ER-negative/HER2-negative MCF10DCIS.com cell line (Miller et al., 2000) recapitulates DCIS-like structures when grown in three-dimensional cell culture models. A model for ER-negative/HER2-negative DCIS also exists in the HMT-3522 series (Rizki et al., 2008). Models for ER-positive *in situ* disease are more limited, however representatives of ER-positive/HER2-positive DCIS and HER2-positive DCIS can be found in the 21T progression series and SUM225 cell line, respectively (Band et al., 1990; Behbod et al., 2009; Forozan et al., 1999; Souter et al., 2010). These cell line models of DCIS may be used in *in vitro* assays probing three-dimensional mammary acinar structure, proliferation, apoptosis and invasion (Debnath et al., 2003; Imbalzano et al., 2009; Lee et al., 2012; Rizki et al., 2008) to annotate functionally potential drivers of progression from DCIS to IBC. In addition, DCIS cell lines have been used in xenograft studies to model progression from DCIS to IBC *in vivo* (Hu et al., 2009; Souter et al., 2010; Lyons et al., 2011), and recently Medina and colleagues (Behbod et al., 2009) developed a 'mammary intraductal' xenograft model, which involves injection of neoplastic cells directly into the mammary ducts

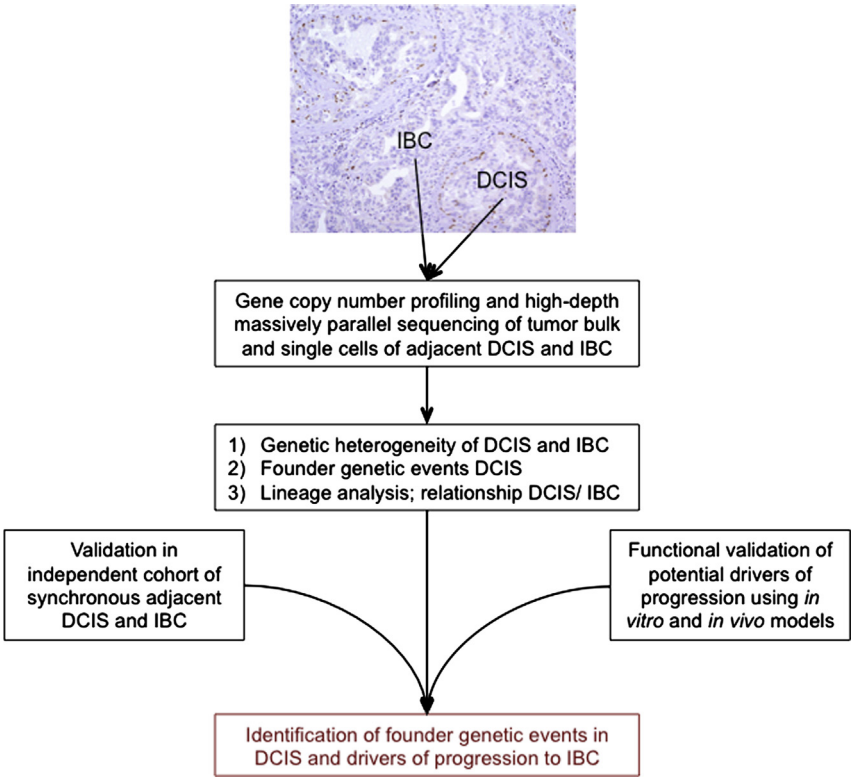

Figure 3 – Schematic of a potential approach for the identification of founder genetic events in DCIS and genetic aberrations that drive progression from DCIS to IBC. DCIS: ductal carcinoma *in situ*; IBC: invasive breast cancer.

via a cleaved nipple, rather than subcutaneous injection to more accurately recapitulate the histology of DCIS. This model, however, still faces the limitations posed by the use of xenografts, including the lack of a competent mouse immune system in the models analyzed and the fact that the tumor–microenvironment interactions between human DCIS cells and mouse myoepithelial and stromal cells may be distinct from those of human DCIS cells, myoepithelial cells and stromal cells, which may be overcome in part by the use of humanized stroma (Kuperwasser et al., 2004). Importantly, however, studies aiming to develop additional *in vitro* and *in vivo* models of DCIS and of the progression from *in situ* to invasive disease, which closely recapitulate the cardinal features of these lesions, are warranted.

6. Conclusion

The discovery of clinically useful biomarkers to differentiate women with DCIS at high or low risk of developing IBC has so far been confounded by the apparent lack of genomic and transcriptomic differences between the two. Recent studies based on medium-throughput sequencing and fluorescence *in situ* hybridization hint at genomic differences and clonal evolution in progression to IBC. In fact, there is evidence to suggest that cancers may follow a Darwinian evolution and that several biological phenomena (e.g. resistance to specific targeted therapies, the process of metastatic dissemination, and progression from *in situ* to invasive disease) may constitute evolutionary bottlenecks. Consequently, the employment of novel technologies, including high depth massively parallel sequencing and single cell analyses, is an important next step to discern the contribution of genomic alterations and Darwinian evolution to the transition from DCIS to IBC.

Conflict of interest

The authors have no conflicts of interest to declare.

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