Review

The diagnosis and management of pre-invasive breast disease

Genetic alterations in pre-invasive lesions

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Published: 9 October 2003

Breast Cancer Res 2003, **5**:313-319 (DOI 10.1186/bcr650)

Abstract

The development of modern molecular genetic techniques has allowed breast cancer researchers to clarify the multistep model of breast carcinogenesis. Laser capture microdissection coupled with comparative genomic hybridisation and/or loss-of-heterozygosity methods have confirmed that many pre-invasive lesions of the breast harbour chromosomal abnormalities at loci known to be altered in invasive breast carcinomas. Current data do not provide strong evidence for ductal hyperplasia of usual type as a precursor lesion, although some are monoclonal proliferations; however, atypical hyperplasia and *in situ* carcinoma appear to be nonobligate precursors. We review current knowledge and the contribution of molecular genetics in the understanding of breast cancer precursors and pre-invasive lesions.

Keywords: atypical ductal hyperplasia, comparative genomic hybridisation, ductal carcinoma *in situ*, lobular carcinoma *in situ*, loss of heterozygosity

Introduction

The multistep model of breast carcinogenesis suggests a transition from normal epithelium to invasive carcinoma via non-atypical and atypical hyperplasia and *in situ* carcinoma. Within the breast, these proliferations are heterogeneous in their cytological and architectural characteristics. The introduction of mammographic screening has led to the increased detection of pre-invasive disease and has highlighted deficiencies in our understanding and classification of such lesions. The morphological classification of pre-invasive lesions of the breast remains controversial and there has been hope that molecular analysis will clarify the uncertainties.

A multitude of methods have been used for the characterisation of pre-invasive breast lesions, including immunohistochemistry, fluorescent *in situ* hybridisation, analysis of loss of heterozygosity (LOH), comparative genomic hybridisation (CGH), and, more recently, cDNA microarrays and proteomics analysis. In this review, we have mainly focused on the genetic abnormalities in pre-invasive lesions of the breast as detected by LOH and CGH analysis (Table 1). The other techniques have been addressed elsewhere in the series.

Ductal carcinoma *in situ*

The analysis of genetic alterations in ductal carcinoma *in situ* (DCIS) has provided new insights in the biology of these lesions. As with invasive carcinoma, abnormalities of chromosomes 1 and 16 have been identified in some of these cases [1]. The CGH method has been modified for paraffin-embedded material and this has allowed studies on archival material and, in particular, the study of pre-invasive disease [2–8]. CGH analysis of DCIS has demonstrated a large number of alterations, including gains of 1q, 5p, 6q, 8q, 17q, 19q, 20p, 20q, and Xq, and losses of 2q, 5q, 6q, 8p, 9p, 11q, 13q, 14q, 16q, 17p, and 22q [2–8]. These alterations are similar to those identified in

ADH = atypical ductal hyperplasia; ALH = atypical lobular hyperplasia; CGH = comparative genomic hybridisation; DCIS = ductal carcinoma *in situ*; HUT = hyperplasia of usual type; LCIS = lobular carcinoma *in situ*; LOH = loss of heterozygosity; NST = no special type.

Table 1

Summary of the genetic abnormalities detected in pre-invasive lesions of the breast[a]

Lesion	Method	Gains[b]	Losses / LOH / AI[b]	Reference
Normal cells	LOH	–	2pter, 16q23.1–24.2, 17q21, 17q24	[17]
	LOH	–	9p, 11p, 13q, 16q, 17p	[56]
	LOH	–	3p24, 11p15.5, 17p13.1	[60]
	LOH	–	1q, 7q, 11p, 16q, 17q	[61]
	LOH	–	11p, 13q	[63]
	LOH	–	3p24.3	[64]
HUT	CGH	No	No	[6]
	LOH	–	2pter, 2q35, 4q25, 6qter, 8p, 9p, 11p15, 11q23, 13q13, 14q24, 16q21, 17p13, 17q11, 17q21, 17q25	[13]
	CGH	1q+, 1q32–42+, 12+, 17q21+, 20+	8-, 8p12-pter-, 9-, 10p-, 11q14-qter-, 18-, X-, 16q-, 17p-, 20p-	[54]
	LOH	–	16q, 17p, 17q	[55]
	LOH	–	9p, 11p, 13q, 16q, 17q	[56]
	CGH	13q	1p, 16p, 17q, 19p, 22q	[57]
ADH	LOH	–	2pter, 2q35, 6qter, 8p, 9p, 11p15, 11q23, 13q13, 14q24, 16q21, 17p13, 17q11, 17q21,	[13]
	LOH	–	17q25, 16q, 17p	[52]
	LOH	–	8p, 16q, 17q	[53]
	CGH	1q, 10, 16p, 8q21-qter, 14q	3q11-q21, 8p12-pter, 16q,20, 11q12-13, 16q 16q, 20p, 16q, 17p, 21q11-q21, 16q, 17p	[54]
ALH/LCIS	CGH	1q, 1q21-q32, 1q25-qter, 8p11-p12, 12q14-q21	7p, 8p, 8p21-pter, 12q24, 16q, 17p, 13q12-q21; 16q; 17p12-p13	[31]
	CGH	6q	16p, 16q, 17p, 22q	[32]
Columnar cell change /clinging ca	LOH	–	2p, 3p, 11q, 11q, 16q, 16q, 17q, 17q	[60]
DCIS[c]	See text	See text	See text	See text

–, none. [a]For LOH analyses, only those studies in which more than one chromosomal arm was evaluated were included in the table. [b]All chromosomal gains and losses reported in the cited studies (references) were included. For the frequency of each genetic abnormality, please see text and cited references. [c]DCIS of different grades harbour distinct chromosomal abnormalities. Genetic abnormalities of nearly all chromosomal arms have been reported in high-grade DCIS. See text for details. ADH, atypical ductal hyperplasia; AI, allelic imbalance; ALH/LCIS, atypical lobular hyperplasia/lobular carcinoma *in situ*; Columnar cell change/ clinging ca, columnar cell change/clinging carcinoma; DCIS, ductal carcinoma *in situ*; HUT, hyperplasia of usual type; LOH, loss of heterozygosity.

invasive carcinoma, adding weight to the idea that DCIS is a precursor lesion.

Several lines of evidence support the concept that different types of DCIS show different genetic alterations, suggesting that there may be multiple pathways for the evolution of DCIS [4,6,8,9]. Alterations at 16q are much more frequent in low-grade DCIS than in high-grade DCIS, in which alterations at 13q, 17q, and 20q are more frequent [4,6,7,10]. Similar findings in invasive carcinomas of low and high grade also support the idea that low-grade and high-grade lesions develop through distinct pathways rather than by dedifferentiation [4,6,7,10]. With the use of microdissection techniques to isolate small microscopic lesions, loss of heterozygosity (LOH) has also been investigated in pre-invasive disease [11–17]. O'Connell and colleagues [11] studied pre-invasive lesions using a variety of chromosomal markers and showed that 50% of the proliferative lesions and 80% of the DCIS shared their LOH patterns with invasive carcinoma. Stratton and colleagues [12] studied cases of DCIS associated with invasive carcinoma and cases of 'pure' DCIS without an invasive component using a limited set of microsatellite markers on chromosomes 7q, 16q, 17p, and 17q. They found a similar frequency of LOH in both subsets of DCIS to invasive carcinoma, providing further strong evidence

that DCIS is likely to be a precursor of invasive carcinoma. Several other reports corroborating these seminal studies have been published [13–20].

c-*erbB2* (*Her-2/neu*) protein has been identified in a high proportion (60–80%) of DCIS of high-nuclear-grade comedo type but is not common in the low-nuclear-grade forms. Allred and colleagues [21] showed that the expression is higher in invasive carcinoma associated with DCIS than in those without DCIS. This oncogene is very rarely overexpressed in classic lobular carcinoma *in situ* (LCIS) and its overexpression has been occasionally observed in cases of pleomorphic lobular carcinoma *in situ* [22,23]. There is no evidence that c-*erbB2* is amplified or overexpressed at the protein level in benign proliferative breast diseases or atypical ductal hyperplasia (ADH) [24], which may suggest that c-*erbB2* is important in the transition from a 'benign' to a 'malignant' phenotype. The difference in frequencies of expression in *in situ* and invasive carcinoma remains a mystery. A number of hypotheses have been advanced, suggesting either that the expression is switched off during invasion or that many c-*erbB2*-positive DCIS do not transform to invasive malignancy. Expression of p53 protein has been demonstrated using immunohistochemistry in high-nuclear-grade DCIS (comedo type) [25]. The mechanism may be gene mutation, but this has been confirmed in only some cases. Like c-*erbB2*, p53 protein expression is rare in LCIS and has not been demonstrated in atypical ductal hyperplasia or other benign proliferative disease [26]. Done and colleagues [27] demonstrated that p53 mutations found in DCIS and associated invasive cancer were absent from benign proliferative lesions from the same breast.

In summary, a considerable body of evidence indicates that DCIS, particularly of high grade, shares many molecular genetic alterations with invasive carcinoma [4–8,14,15]. Therefore, high-grade DCIS should be considered a direct precursor of invasive carcinoma. Moreover, gain of chromosome 1q and loss of 16q, which are highly prevalent in low-grade DCIS, are frequently found in tubular carcinoma and in tubular, tubulolobular, lobular, and grade 1 invasive ductal carcinomas [4,6,8,28], suggesting that low-grade DCIS is also a direct precursor for certain types of breast carcinomas.

Lobular carcinoma *in situ*

Lobular carcinoma *in situ* of the breast is an uncommon lesion with a distinctive appearance. It is classically composed of discohesive cells with small, monomorphic, hyperchromatic nuclei; however, a pleomorphic variant has been described [23,29]. It is occasionally confused with DCIS of low-grade, solid type; however, epidemiological studies show that its biological behaviour and clinical implications are quite different from those of DCIS. It is usually an incidental finding and is not visible on mammography [29]. The lesions are multifocal and bilateral in a high proportion of cases [29]. The majority of cases are diagnosed in patients aged between 40 and 50 years, a decade earlier than DCIS. Approximately one-fifth of the cases will progress to invasive cancer over a 20- to 25-year follow-up period [29]. Although invasive ductal carcinomas, especially of tubular type, do occur after LCIS, most cases associated with LCIS are infiltrating lobular carcinoma [29]. It has been said that the risk is equal for the two breasts [30]; however, there are data to suggest that the risk is skewed in favour of the ipsilateral breast [29,31]. Despite these thorny issues, the epidemiological and pathological features of LCIS have raised questions about its biological nature, and some still consider it a 'marker of increased risk' rather than a true precursor of invasive carcinoma.

In our laboratories, we have carried out CGH analysis on LCIS and atypical lobular hyperplasia [32]. Loss of material from 16p, 16q, 17p, and 22q and gain of material from 6q have been found at similar high frequencies in both LCIS and atypical lobular hyperplasia. Losses at 1q, 16q, and 17p have also been seen in invasive lobular carcinomas [8,33]. LOH data in LCIS are also limited but do demonstrate a similarity between LCIS and infiltrating lobular carcinoma [34,35].

E-cadherin is a candidate tumour suppressor protein coded by a gene on 16q22.1, which is involved in cell–cell adhesion and in cell-cycle regulation through the β-catenin/Wnt pathway [36]. The majority of invasive ductal carcinomas of no special type (NST) usually exhibit positive staining by immunohistochemistry, whereas the overwhelming majority of invasive lobular carcinomas are negative [37–39]. E-cadherin truncating mutations associated with loss of the wild-type allele (LOH at 16q) have been observed in LCIS and invasive lobular carcinomas [38,40,41]. Berx and colleagues [40] failed to identify any truncating mutations in invasive ductal carcinomas of NST or medullary carcinomas; similar findings were recently reported by Roylance and colleagues [39], who demonstrated lack of E-cadherin mutations in 44 low-grade ductal carcinomas of NST. E-cadherin is expressed in normal epithelium and in most of the cases of DCIS, but staining is rarely seen in LCIS [23,38,39,42–46]. Based on this differential expression of E-cadherin in LCIS and DCIS, some authors have advocated the use of antibodies against E-cadherin as an adjunct marker for the differentiation of LCIS from DCIS [23,44–47].

In addition, Vos and colleagues [41] have demonstrated the same truncating mutation in the E-cadherin gene in LCIS and the adjacent invasive lobular carcinoma. The data provide strong evidence for the role of the E-cadherin gene in the pathogenesis of lobular lesions and also support the hypothesis of a precursor role for LCIS.

Although E-cadherin germline mutations have been implicated in the pathogenesis of familial diffuse gastric carcinoma, there are only anecdotal case reports of lobular carcinoma arising in patients with germline alteration in the gene [36]. In contrast, Rahman and colleagues [46] failed to find any pathogenic E-cadherin germline mutations in 65 patients with LCIS and positive family history of breast carcinoma, thus suggesting that E-cadherin is unlikely to act as a susceptibility gene for LCIS.

Atypical ductal hyperplasia

ADH is a controversial lesion, which shares some but not all features of DCIS. It poses considerable difficulties in surgical histopathology. In order to address this problem, Page and Rogers [48] laid down criteria for the diagnosis of this entity. Rosai [49] in his study had demonstrated a high interobserver variability in the diagnosis of ADH. However, a subsequent study by Schnitt and colleagues [50], in which the pathologist used Page's criteria, showed an improvement, with complete agreement in 58% of cases. Within the UK National External Quality Assessment Scheme [51], agreement even among experienced breast pathologists has been low. Lakhani and colleagues [52] demonstrated that LOH identified at loci on 16q and 17p in invasive carcinoma and DCIS is also present in ADH with a similar frequency. Similar results were reported by Amari and colleagues [53]. O'Connell and colleagues [13] studied 51 cases of ADH at 15 polymorphic loci and found LOH at at least one marker in 42% of the cases. The studies demonstrate that morphological overlaps are reflected at the molecular level and raise questions about the validity of separating ADH from DCIS. CGH analysis of nine cases of ADH revealed chromosomal abnormalities in five of them [54]. As expected, owing to the morphological overlap with low-grade DCIS, losses of 16q and 17p were the most frequent changes found in ADH [54].

Hyperplasia of usual type

O'Connell and colleagues [13] demonstrated that LOH at many different loci can be identified in hyperplasia of usual type (HUT), with frequencies ranging from 0 to 15%. These figures are similar to those of Lakhani and colleagues [55], who reported data in non-atypical hyperplasia (HUT) dissected from benign breast biopsies. LOH was identified at frequencies ranging from 0 to 13% at a locus on 17q. These frequencies are much lower than those identified in DCIS and ADH (range 25–55%). In the series reported by Washington and colleagues [56], 4 of 21 HUTs showed LOH in one to five loci. LOH at 16q (three cases), 9p (three cases), and 13q (two cases) were the most frequent findings [56]. Although CGH analysis of HUTs has demonstrated that the majority of these lesions harbour no chromosomal abnormalities [6,55–57], the picture dramatically changes when they are associated with ADH or DCIS [54]. In this setting, most lesions show losses of 16q and 17p [54]. In our view, the majority of HUTs do not appear to be precursors of DCIS and IDC, but the precursor potential of a small subset of these lesions cannot be excluded based on the reports of synchronous HUT and invasive breast cancer sharing a common genetic lineage [13].

A word of caution should be voiced, as in the majority of the studies published to date, the contamination of HUTs with neoplastic cells of ADH and DCIS could not be excluded. This issue was recently addressed in a study published by Jones and colleagues [57], in which the authors analysed 14 cases of bilateral HUTs (28 lesions) by CGH. To avoid the inclusion of dubious lesions or contamination of HUTs with neoplastic cells, the authors defined HUTs according to the criteria proposed by the Pathology Working Group on Behalf of the Breast Screening Program and immunohistochemically with antibodies against cytokeratins 5/6. In that study [57], 18 of 28 lesions from 10 of 14 patients harboured chromosomal abnormalities, which ranged from 0 to 5, with a mean of 1.6. The most common genetic alterations were gains of 13q and losses at 1p, 16p, 17q, 19p, and 22q. When paired HUTs from the same patients were compared, only five concordant genetic abnormalities were observed, and only one of these appeared more than once (loss of 17q, in two cases). These findings corroborated those reported by O'Connell and colleagues [13], who evaluated multiple foci of HUT affecting the same breast (53 breasts) and found that only 15% of the lesions within the same breast shared their LOH phenotype. Altogether, owing to limitations imposed by the currently available methodology, it seems that a relatively small proportion of HUTs are monoclonal, neoplastic proliferations, but the evidence in support of HUT as a precursor of DCIS and IDC is still weak.

Columnar cell lesions

Columnar cell lesions have been a major source of confusion among breast pathologists, first because they have been reported under several different names, including columnar alteration of lobules, blunt duct adenosis, *metaplasie cylindrique*, cancerisation of small ectatic ducts of the breast by ductal carcinoma *in situ* cells with apocrine snouts [58], columnar alteration with prominent apical snouts and secretions [59], and clinging carcinoma *in situ* [60]. These lesions represent a spectrum that ranges from columnar cell alteration in luminal cells to ADH and flat/clinging DCIS. Regardless of the fact that there are several lines of evidence showing an association with tubular carcinoma [59,60], only one paper has addressed the genetic abnormalities in these lesions [60]. Moinfar and colleagues [60] demonstrated that 77% of columnar cell lesions (either with or without atypia) harbour chromosomal abnormalities at least in one locus and the most frequent loci of LOH were 11q21–23.2, 16q23.1–24.2, and

3p14.2 [60]. It is noteworthy that 16q and 11q are frequently lost in tubular carcinomas [28,60]. More interestingly, these authors [60] have also shown that otherwise luminal cells with mild nuclear atypia lining ducts at the vicinity of columnar cell lesions may also have loss of genetic material in up to 6% of the cases.

Normal tissues

Over the past few years, seven studies have also demonstrated that LOH identified in invasive carcinoma is already present in morphologically normal lobules [17,36,56,61–64]. Lakhani and colleagues [63] demonstrated that LOH identified in normal breast epithelial cells is seen independently in luminal and myoepithelial cells, suggesting a common precursor cell for these two types of epithelial cell. Even more thought provoking is the data published by Moinfar and colleagues [17], who demonstrated the presence of concurrent and independent genetic alterations in normal-appearing stromal and epithelial cells located either in the vicinity of or at a distance from the foci of DCIS or IDC. The extent and frequency of alterations and their significance in the multistep carcinogenesis remain unknown at present. It should be noted that in breasts without malignant changes, genetic alterations in normal cells are rather infrequent, subtle, and fairly random [6]. Conversely, one paper has demonstrated that normal lobules and adjacent *in situ* carcinomas show concordant genetic alterations [17], and another suggested that LOH in lobular units in terminal ducts in the normal breast is predictive of local recurrence [64].

Conclusion

Molecular biology and genetics have provided new insights for the comprehension of biology of pre-invasive lesions of the breast. CGH and LOH studies have partially corroborated the multistep model of breast carcinogenesis by demonstrating similar chromosomal abnormalities in ADH and DCIS. More interestingly, these findings challenge the concept of HUT as a precursor of breast cancer and suggest that columnar cell alteration may be a peculiar form of pre-invasive lesion and, possibly, a precursor of low-grade invasive ductal carcinomas of the breast. These techniques have also demonstrated that different types of *in situ* breast carcinoma harbour different chromosomal abnormalities, and these findings may reflect the involvement of different pathways in the multistep model of breast carcinogenesis.

We are still in the early phase of molecular analysis of pre-invasive lesions. Dramatic advances in the understanding of these lesions may be expected with the development of more flexible microdissection systems (suitable for fresh/frozen samples) and the advent of high-throughput-technology methods suitable for the evaluation of paraffin-embedded tissues (e.g. CGH arrays).

This article is the eighth in a review series on *The diagnosis and management of pre-invasive breast disease – current challenges, future hopes*, edited by Sunil R Lakhani.

Other articles in the series can be found at http://breast-cancer-research.com/articles/review-series.asp?series=bcr_Thediagnosis

Competing interests

None declared.

Acknowledgements

JSRF is the recipient of the Gordon Signy International Fellowship Award of the World Association of Societies of Pathology and Laboratory Medicine (WASPALM) and is partially supported by a PhD grant (ref. SFRH/BD/5386/2001) from the Fundação para a Ciência e a Tecnologia (FCT), Portugal.

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