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Intermediate histological effect markers for breast cancer

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The evidence that ductal carcinoma of the breast (DCIS) is an obligate precursor of invasive breast cancer and thus qualifies as an intermediate effect marker in chemoprevention research is reviewed. Much of the evidence on the natural history of DCIS has been derived from the era before the introduction of mammographic screening. Thus it may not be applicable in the present situation when women are likely to be under mammographic surveillance in chemoprevention trials. Further, the data that are becoming available from breast screening trials suggest that at least over follow-up periods now exceeding a decade, detection and treatment of DCIS has no impact on subsequent incidence of breast cancer. Although there are some indications that other biomarkers of malignancy are expressed similarly in DCIS and invasive cancer of similar grade, this evidence may not be sufficient to allow exclusive reliance on DCIS as an intermediate-effect marker in chemoprevention research.

Introduction

It is generally believed that ductal carcinoma *in situ* (DCIS) is a pre-invasive neoplastic lesion in the breast, and that possibly atypical hyperplasia of the breast is a preneoplastic lesion. Indeed, chemoprevention trials are already being based on women with DCIS, in the belief that this is a valid surrogate end-point biomarker (Kelloff *et al.*, 2000). However, the evidence that women with biopsies indicating atypical hyperplasia or DCIS have a greater risk of breast cancer has largely come from long-term follow-up of cohorts of women whose biopsies were originally considered to represent benign (fibrocystic) disease (Page *et al.*, 1982, 1985). It is not clear from these studies whether the histological abnormality indicated a marker of increased risk of breast cancer, or whether it was truly a precursor lesion that if left untreated could eventually progress to invasive cancer. Part of the difficulty is that the histological abnormality was found in the original biopsies, and there was no information on whatever abnormality (if any) — either precursor lesion or early invasive cancer — had been left behind. Suspicion that the subsequent occurrence of invasive cancer in patients who developed DCIS came from a residual abnormality is derived from the fact that the risk of invasive cancer seemed to be restricted to the breast from which the original biopsy was obtained, not the contralateral breast. This is quite different from the related lesion, lobular carcinoma *in situ*, or lobular neoplasia, where the risk of subsequent breast cancer is in both breasts (Lagios & Page, 1998). Hence lobular carcinoma *in situ* seems to represent a marker of risk, not a precursor lesion, and will not be considered further here. Similarly, as atypical hyperplasia should probably be regarded as a preneoplastic lesion rather than as a defined precursor on the pathway to cancer, it will also not be considered in detail in this chapter.

Nomenclature of DCIS

Although different nomenclatures have been in vogue, DCIS has been classified in Europe as poorly differentiated, intermediately differentiated or well differentiated (Holland *et al.*, 1994). This classification is based on the degree of nuclear differentiation and on cellular polarization around intercellular spaces or towards a duct lumen. In North America, in the Van Nuys classification, DCIS is initially subdivided into those lesions with high nuclear grade or non-high nuclear grade, and the latter are then subdivided into those with or without necrosis (Silverstein *et al.*, 1995). This results in three prognostically dissimilar groups. Alternatively, DCIS has been classified as comedo,

intermediate and non-comedo (solid, cribriform or papillary) (Lagios & Page, 1998). In comedo-type DCIS necrosis is extensive and the nuclear grade is high; in intermediate-type DCIS necrosis is focal or absent and nuclear grade is intermediate; in non-comedo-type DCIS, necrosis is absent and nuclear grade is low. The risk of subsequent invasive cancer is believed to be highest for the comedo type or high-grade (poorly differentiated) DCIS.

Markers of carcinogenesis, and relevance to natural history of DCIS

The use of digital image analysis of the DNA content of DCIS and invasive intraductal carcinomas associated with them showed a close concordance of DNA-ploidy and S-phase content (Fisher & Siderits, 1992). However, whether or not DCIS is directly on the pathway to cancer, and is in practice an obligate precursor to invasion, can only be inferred indirectly, given the process of biopsy that is required for its diagnosis. One approach to resolving this uncertainty is to assess whether biomarkers of neoplasia which can be identified in invasive breast cancers (logically ductal type in the majority) are also expressed in DCIS. Weinstat-Saslow *et al.* (1995) have shown that overexpression of cyclin D mRNA occurs equally in DCIS and invasive cancers, and can be used to distinguish both from non-malignant breast lesions. They found overexpression of cyclin D mRNA in 76% of 37 non-comedo DCIS, in 87% of 23 comedo DCIS and in 83% of 12 invasive breast carcinomas. These percentages are not statistically significantly different from each other. However, they found cyclin D mRNA overexpression in only 18% of 11 lesions of atypical hyperplasia and 18% of 11 benign lesions interpreted as showing no increase in risk. These percentages were significantly different from those of DCIS and invasive cancer.

Buerger *et al.* (1999) analysed specific chromosomal alterations of 38 cases of DCIS and six associated invasive breast cancers by means of comparative genomic hybridization. They found losses of 16q material almost exclusively in well and intermediately differentiated DCIS. A higher frequency of amplifications (17q12, 11q13) was found in poorly differentiated DCIS. When DCIS was adjacent to invasive carcinoma, a similar genetic pattern was seen in the DCIS and invasive component.

A slightly different approach to the same issue was taken by Dublin *et al.* (1999). These authors re-evaluated material from 98 cases originally diagnosed as minimal (< 10 mm) invasive breast cancers. Of these, 28 were found to be predominantly invasive, 48 predominantly DCIS and 22 DCIS without evidence of invasion. In the predominantly invasive group, the infiltrative component was usually > 5mm, was low-grade, and associated with well differentiated DCIS. Expression of the markers Ki-67, c-erbB2 and p53 was generally low, and that of ER, PR and Bcl-2 high. In contrast, the predominantly DCIS group had a much smaller high-grade invasive component, usually with poorly differentiated DCIS. In these, expression of Ki-67, c-erbB2 and p53 was generally high, and ER, PR and Bcl-2 low. The actuarial survival of the predominantly invasive group was 100% beyond 10 years, of the predominantly DCIS group over 80%; the difference was not statistically significant. In this study it seemed that the high-grade lesions, whether invasive or DCIS, had a similar spectrum of markers indicating, but not demonstrating in practice, poor prognosis, while the low-grade invasive cancer and DCIS also were similar. However, this study also could be interpreted as suggesting that 'invasion', in what would otherwise be regarded as a predominantly DCIS lesion, is of little or no prognostic importance. This somewhat startling conclusion suggests that for lesions that are predominantly DCIS, whether or not cells have penetrated the basement membrane is unimportant. The absence of significance of this generally regarded criterion of progressive malignancy would be explained if somehow the cells forming the outer levels of DCIS manufactured their own basement membrane. Indeed, there are other features of some cases of DCIS, especially those with associated periductal fibrosis, that suggest that DCIS is really a form of good-prognosis invasive cancer (Naresh & Borges, 1996). Whatever the true explanation, it does seem that many cases with minimal 'invasion' by standard criteria are qualitatively different from usual invasive cancer, thus adding to the doubts that DCIS is a precursor of true invasive cancer.

Whether or not recurrences of DCIS occur that are truly new disease or simply recurrent slowly growing lesions has recently been evaluated. Comparative genomic hybridization was used by

Waldman *et al.* (2000) to compare chromosomal alterations in 18 initial DCIS lesions (occurring in the absence of invasive cancer) and subsequent recurrences of DCIS in the same breast. They found a high degree of concordance of chromosomal alterations in all but one of the initial/recurrence pairs, suggesting that the large majority of the recurrences were caused by residual DCIS left at the time of the primary surgery. The most common chromosomal alterations noted were gains involving 17q and losses involving 8p and 17p.

In commenting on this study, Fisher & Fisher (2000) reported that in their experience with about 10 000 cases of DCIS associated with invasive cancer, unequivocal microscopic extension of DCIS through its basement membrane into the surrounding stroma had rarely been observed. They also noted that although about 40% of recurrences of DCIS in the same breast were invasive, the survival of such patients was 98%, again suggesting a qualitatively different natural history from classic invasive ductal carcinoma. This seems to be an example of length bias.

DCIS detected by screening

Recently, screening programmes for breast cancer have led to the diagnosis of much larger numbers of DCIS, as the calcification associated especially with comedo types of DCIS leads to their detection on mammography, biopsy and excision. The detection of DCIS is believed by many to be one of the benefits derived from breast cancer screening. Indeed, aggressive screening for what was then called minimal breast cancer used to be strongly advocated in the belief that only by the detection of such lesions would breast cancer mortality be reduced (Moskowitz *et al.*, 1976). Minimal breast cancer as then defined consisted of two components, invasive breast cancers < 10 mm in size and DCIS. Data allowing assessment of their contribution to the reduction in breast cancer potentially achieved by screening have recently become available from the long-term follow-up of the Canadian National Breast Screening Study among women aged 50–59 years on entry (CNBSS 2) (Miller *et al.*, 2000). This study was designed to assess whether annual two-view mammographic screening together with annual screening by breast physical examinations and the teaching of breast self-examination (the MP group) resulted in a greater breast cancer mortality reduction than screening by breast physical examinations and the teaching of breast self-examination alone (the PO group). Each group comrised a total of just under 20 000 women, with identical distribution of risk factors for breast cancer (CNBSS was an individually randomized trial, randomization being conducted within five-year age strata and centre). Of the 267 invasive breast cancers detected on screening in the MP group, 48 were < 10 mm in size, compared with only six of 148 in the PO group. Further, in addition, 71 *in situ* breast cancers were detected in the MP group but only 16 in the PO group. However, no reduction in breast cancer mortality was found by the addition of mammography (a cumulative rate ratio of 1.02, 95% CI 0.78–1.33). Thus the greater detection of 'minimal' breast cancers in the MP group (an excess of 97) had no impact on breast cancer mortality. Further, there was no evidence that the detection of the *in situ* cancers resulted in a reduction in breast cancer incidence, the cumulative numbers of invasive breast cancers (including those ascertained after the end of the 4–5-year screening period) were 622 in the MP group and 610 in the PO. The data from the 50 000 women aged 40–49 years on entry to CNBSS 1 are currently under analysis. Once again, there was an excess of *in situ* cancers diagnosed in the mammographic screening group compared with the usual care group, but no indication of a reduction in breast cancer incidence over the 11-year follow-up period.

Similar data have not been published from the other breast screening trials, but at present there seems to be no evidence that detection of breast cancer precursors is of any value in screening.

Boyd *et al.* (this volume) postulate that mammographic density may be an exposure marker for breast cancer, but cannot be regarded as an intermediate marker. However, Boyd *et al.* (1992, 2000) derived evidence that mammographic density is associated with atypical hyperplasia and DCIS. In a special study of women aged 40–49 years who had enrolled in CNBSS 1, it was found that women with the most extensive densities had a 9.7 times greater risk of being detected with carcinoma *in situ* or atypical hyperplasia than women with no mammary density.

Discussion

Part of the difficulty in determining the role of DCIS in chemoprevention may be that with mammographic screening, a new spectrum of disease has come to light that would have been largely undiagnosed in the absence of screening. This has contributed to the confusion as to what these lesions truly represent, and even whether it is appropriate to include the term 'carcinoma' within a terminology where no precise guidance can be provided on eventual prognosis (Foucar, 1996). It cannot therefore be assumed that the natural history data derived from the follow-up of women diagnosed in a pre-screening era applies to those now detected by screening. As already indicated, some of the presumed precursors may not be precursors at all, but simply markers of increased risk. It is quite possible that the true invasive cancer precursors, with atypical epithelial hyperplasia and incipient invasion, are largely not detectable with current screening methods. Indeed, insufficient cases of DCIS are detected by mammography to account for the numbers of invasive cancers that occur. This may be because some high-grade cancers have a transitory *in situ* phase with rapid progression to invasion, thus not allowing time for their detection as DCIS (Barnes *et al.*, 1992). The DCIS that is detectable may either be a marker of atypical epithelial hyperplasia or invasive cancer elsewhere in the same breast, an indolent lesion that progresses only slowly, or the end stage of a process well recognized for cancer of the cervix, namely the regression of the majority of the precursors that are detectable.

That detection of precursors will result in overtreatment of many patients not destined to develop invasive cancer has been well demonstrated for cancer of the cervix, and seems likely to be true for the breast also. This makes it essential that there should not be overtreatment of these lesions, as appears to have occurred in the CNBSS, for example (Miller, 1994), and that great caution be exercised in utilizing them as end-points for the evaluation of chemoprevention. It is quite possible that studies that are conducted of chemoprevention with DCIS diagnosed by mammography as an end-point may be largely irrelevant to the prevention of invasive cancer. An indication that the wrong lesions could be prevented from occurring even when invasive cancer is used as the end-point comes from the NSABP trial of tamoxifen for chemoprevention of breast cancer (Fisher *et al*, 1998). The spectrum of estrogen-positive, relatively small breast cancers that failed to occur in the tamoxifen-treated group compared with the control group suggests that these were lesions with good prognosis, with a low probability of resulting in death. This is precisely the spectrum of the lesions that were detected early in CNBSS 2, but whose earlier detection did not result in any indication of reduction in breast cancer mortality (Miller *et al.*, 2000). It is relevant that the participants in the NSABP trial were monitored by annual mammography; the follow-up of CNBSS 2 suggests that such lesions represent a classic example of length-biased sampling.

An alternative approach to using DCIS in chemoprevention research would be to select people with high-grade lesions for study of a potential chemopreventive agent, with invasive cancer as the outcome measure. Thus DCIS would be being used as an exposure or acquired susceptibility marker. However, it seems probable that the majority of women destined to develop and die of invasive cancer may be missed by such an approach, and thus any inferences derived from such a study would be subject to considerable caution.

Conclusions

DCIS detected by mammography, and currently being considered for use as an end-point for chemoprevention drug development trials, may not be an obligate precursor of invasive breast cancer. It may even be a subsegment of invasive breast cancer with very low progression potential and therefore with a very good (and non-typical) prognosis. As such, any conclusions derived from the use of DCIS as an end-point in chemoprevention trials may be in error. Research is needed into the natural history of DCIS diagnosed by mammography. Methods to detect the true obligate precursors of invasive breast cancer are urgently needed.

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