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Review

Premalignant Breast Lesions: Role for Biological Markers in Predicting Progression to Cancer

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Biological markers associated with *in situ* carcinoma and atypical intraductal hyperplasia in the breast are examined to help in identifying a subgroup of premalignant lesions whose natural history may be influenced by epigenetic factors. The biomarkers may be used as indices in clinical trials aiming to assess the effect of weight reduction, dietary intervention or hormone replacement therapy on the risk of progression to invasive breast cancer. In the current state of knowledge, the expression of oestrogen receptors, p53, *bcl-2* and *HER-2 neu* oncogenes and the Ki-67 index of proliferative activity, are the most useful biomarkers for this purpose. *In situ* carcinoma of the breast manifests a variety of morphological phenotypes with specific biological characteristics. There is evidence that only a proportion of premalignant lesions are committed to progression to invasive cancer while other lesions undergo spontaneous regression at the time of the menopause. Cross-cultural studies suggest that it is the late-stage epigenetic promoting factors which are responsible for the high incidence of postmenopausal breast cancer in Western women. Obesity in middle life and the Western diet favour the development of hyperinsulinaemic insulin resistance, and the metabolic–endocrine effects of its concomitants may promote mammary carcinogenesis around the time of the menopause and increase the incidence of invasive cancer after the menopause. Because biomarker changes in premalignant lesions are nearer in time to these promoting influences, they could provide intermediate endpoints for testing the hypothesis. © **1999 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

THE INCIDENCE of breast cancer in Western women is approximately five times that in Asian women and is predominantly postmenopausal in its age distribution. In Western populations the age-specific rate rises progressively even into old age, whereas in Asian countries it increases only up to the age of 50 years and then falls or remains static [1]. When low-risk races migrate to Western countries their incidence of postmenopausal breast cancer rises progressively in successive generations [2].

Carcinogenesis in mammary tissue is thought to involve a series of somatic genetic alterations in the mammary duct cells. Histological field changes have been identified in the extratumoral tissue of cancer-containing breasts and both atypical ductal hyperplasia and *in situ* carcinoma are regarded as premalignant lesions [3]. Ethnic populations with a low breast cancer risk show a lower prevalence of premalignant lesions in the general population than do groups with a high breast cancer risk [4, 5], suggesting a similar aetiology for both lesions. However, cancerous breasts in low-risk populations show a *higher* prevalence of premalignant lesions than do high-risk groups, both in younger and older women [6]. Taken together with the migrant data, it suggests that in high-risk countries, it is the late-stage promoters of carcinogenesis which contribute to the high rates of postmenopausal invasive breast cancer [6].

Biological markers of progressive carcinogenesis, including oncogenes, growth factors and steroid receptors, have recently been quantified in premalignant lesions as well as in

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invasive breast cancer [7, 8]. The review examined the possibility of integrating the molecular markers into clinical management. Nipple aspirates, and fine needle or core aspirates of breast tissue, may be able to provide a surrogate endpoint to determine the effect of hormone replacement therapy, weight reduction or dietary intervention on progression to invasive breast cancer [9, 10].

EPITHELIAL HYPERPLASIA AND *IN SITU* CARCINOMA

Intraductal hyperplasia without atypia is more frequent with increasing age. It is found in 5–20% of young women but in 20–50% of middle aged women [6]. While it is associated with a 2-fold increased risk of subsequent invasive breast cancer, the risk is 3-fold in the presence of atypia [11]. There is no evidence that the frequency of intraductal hyperplasia is related to genetic factors and it is not increased in women with a family history of breast cancer. However, the two factors act synergistically on breast cancer risk [12].

Atypical intraductal hyperplasia is found in approximately 10% of screened women [13] and its frequency in cancerous breasts is reported to peak at the age of 40 years and then falls to a low level at the age of 55 years [14]. Screening mammography shows a peak incidence for ductal carcinoma *in situ* (DCIS) at the age of 45–50 years followed by a steady decline [15, 16]. Histopathology also shows that the incidence of DCIS in mastectomy specimens declines after the menopause [17].

Several biological markers alter according to the phase of mammary carcinogenesis. Proliferative activity is clearly increased with progression of carcinogenesis and is currently measured by immunoassay of Ki-67 labelling [18]. It is, however, recognised that even with high proliferative activity, lesions can enter a dormant state if the rate of cell death is high. Among the regulators of genetically programmed cell death (apoptosis) in normal and neoplastic tissue are the p53 suppressor gene [19] and the *bcl-2* oncogene [20].

An abundance of mutated p53 protein commonly occurs in a variety of tumours including invasive breast cancer. The overexpressed mutated p53 protein is not found in normal breast epithelium or ductal hyperplasia unless there is evidence of atypia [21], but it is recognised in a minority of DCIS specimens especially in the comedo type [22, 23]. This may represent a subset in which apoptosis is no longer controlled, leading to enhanced tumour cell survival. Mutated p53 found in extratumoral foci of DCIS in cancerous breasts is similar to that in the tumour [21] and it is relevant that the labelling index for apoptosis is reduced in the extratumoral tissue of cancerous breasts compared with breast tissue from normal controls [24].

The expression of oestrogen receptor protein (ER) in normal breast epithelium is associated with increased breast cancer risk and may reflect abnormally increased responsiveness to oestrogen [25]. However, in premalignant lesions, ER expression is not consistently related to proliferative activity. Higher proliferative activity is found in ER positive than in ER negative specimens of ductal hyperplasia with atypia but the reverse is seen in DCIS lesions and invasive breast cancer [26]. The role of ER in breast cancer development is thus unclear, but it may be influenced by paracrine activity of growth factors [27].

The expression of various growth factors may change with progression in precursor lesions in the breast. Thus, expression of epithelial growth factor receptor is found in a high proportion of benign breast lesions [28] whilst expression of the closely related *HER-2neu* oncogene increases with progression to DCIS and invasive breast cancer. The expression of insulin-like growth factor also changes with progression and this is discussed in a later section in relation to its interaction with ER activity and possibly also with the p53 gene.

DCIS lesions show considerable morphological and biological heterogeneity [22, 29]. Most of the cribriform-type lesions show high apoptotic activity and p53 expression similar to that of normal breast epithelium, and may be in a steady state with no apparent growth [30]. However, some of the comedo-type lesions show overexpression of mutated p53 and lower expression of ER protein [31]. These are assumed to be immediate precursors of invasive breast cancer.

The proto-oncogene *bcl-2* interacts with p53 and *c-myc* genes in the regulation of apoptosis, and its expression is inversely correlated with mutated p53 expression in mammary carcinogenesis [30]. The expression of bcl-2 progressively decreases with the change from DCIS to invasive breast cancer [22, 23]. Nevertheless, bcl-2 mediates cell division as well as cell death in breast cancer progression and this may explain its persistence in some well-differentiated invasive breast cancers. Expression of the oncoprotein HER-2neu in DCIS lesions increases with increasing grade of malignancy [29, 32] and the presence of extensive necrosis in DCIS is associated with the expression of mutated p53 and HER-2 neu [19, 33].

A recent study on the biological profiles of 74 *in situ* cancers of the breast suggests that biological characterisation can be used to define the natural history of the lesions [7]. The highest positivity for ER was found in cribriform DCIS and lobular carcinoma *in situ* (LCIS). The highest values for mutated p53 and HER-2neu expression and for proliferative activity were in comedo DCIS. Expression of bcl-2 was found in practically all cribriform, non-comedo DCIS and LCIS types.

Another study has compared the levels of biomarkers in invasive breast cancer with those in *in situ* carcinoma and also in mixed invasive/*in situ* carcinoma [8]. Higher positivity for ER was found in cribriform DCIS and in LCIS and the levels were higher in post- than in premenopausal women. Higher positivity for mutated p53 and HER-2neu expression and higher proliferative activity were found in DCIS and invasive cancer, and levels were lower in post- than in premenopausal women. Especially interesting was the finding that in postmenopausal women HER-2neu was expressed less in pure invasive cancer than in mixed lesions. The observation was taken to suggest that not all *in situ* cancers overexpressing HER-2neu (comedo-type DCIS) necessarily progress to invasive breast cancer during a patient's lifetime [8].

HIGH-RISK AND LOW-RISK POPULATIONS

As mentioned above the prevalence of DCIS decreases after the menopause and markers of aggressive growth are less commonly expressed in postmenopausal women [8]. From these observations one would expect less progression to invasive breast cancer after the menopause. However, while apoptosis may be enhanced in a subset of DCIS lesions, they are unlikely to revert to normal growth. It has been suggested that they enter a dormant phase which can be reactivated by epigenetic factors [34]. It is postulated that such reactivation occurs far more frequently in Western than in Asian women

as a result of metabolic–endocrine factors which promote carcinogenesis around the time of the menopause [35]. Data on breast cancer biomarker activity in low-risk populations are scarce. A small series of primary breast cancers in Japanese women has been reported to show a high frequency of p53 mutations and this was suggested as a possible factor in malignant change [36].

Lifestyle factors are likely to contribute to the progression of premalignant lesions to invasive breast cancer. In Japan, the incidence of DCIS is lower than in Western women [1], but in ethnic Japanese women in Hawaii, it is higher than in Western women [37]. Similarly, the incidence of breast cancer in Japanese women in their own country is only 15–25% of that in Western women and is particularly low in postmenopausal women. Yet when they migrate to Hawaii or the U.S.A., breast cancer risk rises with each generation, involves postmenopausal women in particular and is associated positively with increased body mass [38].

It is widely assumed that the fall in oestrogen levels at the time of the menopause is responsible for spontaneous resolution of premalignant lesions [39] and it is postulated that the increased levels of bioavailable oestrogen which are associated with perimenopausal weight gain might maintain stimulation of premalignant lesions [35]. Stimulation will be prolonged as a result of the delayed menopause associated with obesity, the peak age at the natural menopause being 50–54 years for obese women compared with 45–49 years for lean women [40]. Oestradiol levels have been reported 1.71-fold higher in Western women than in Chinese women in the age group 55–64 years [41] and the difference was ascribed to the higher average body weight in Western women.

Obesity increases oestrogen levels in postmenopausal women as a result of increased aromatisation of androgen to oestrogen in excess fat deposits [42]. Obesity is also associated with decreased levels of sex hormone-binding globulin leading to a rise in bioavailable levels of both oestradiol and testosterone [43, 44]. Because the affinity of the globulin is greater for testosterone than for oestradiol, the androgen/oestrogen balance shifts towards androgen. This is thought to directly deposit fat to the abdomen rather than to the femoral-gluteal region [45] (typical gynoid distribution).

The incidence of postmenopausal breast cancer in Western women has increased in recent decades [46]. Although obesity is suspected to be a major factor, the typical Western high-fat diet may also be involved and an association has been shown between increased saturated fatty acid intake and a higher level of ductal atypia [47]. No association has been reported between the fatty acid profile of subcutaneous fat and ductal atypia frequency [48]. Diet and obesity may have independent effects on mammary carcinogenesis as suggested by observations on Japanese and Caucasian women in Hawaii [37]. They may also account for the relatively low incidence of breast cancer in obese postmenopausal Hispanic women in the U.S.A. compared with their non-Hispanic neighbours [49].

In Western women, the proportion of ER positive breast cancers and also the actual ER level have increased in the past 30 years [50]. Later age at first childbirth or the use of oral contraceptives and hormone replacement therapy may have contributed [51], but increasing obesity in the Western population may be a major factor [52]. A positive correlation has been shown between the presence of obesity and ER positive breast cancer, especially in postmenopausal women [53]. In postmenopausal Japanese women, breast cancers show a lower ER positivity than in Western women but this does not apply if they are obese [54].

The normal breast epithelium in European women shows a higher rate of ER expression than that in non-European women (19 versus 4%) [55] and it has been postulated that the ER status of normal breast tissue may predict breast cancer risk [25]. It is however difficult to explain why comparisons of serum oestrogen levels in ER positive versus ER negative cases have produced inconsistent results [27]. The following experimental observations suggest that the expression of ER is interlinked with that of insulin-like growth factor receptor in relation to the stimulation of proliferative activity of breast cancer cells [56].

INTERACTION BETWEEN ER AND INSULIN-LIKE GROWTH FACTOR RECEPTOR

Weight gain in the years leading up to the menopause is commonly associated with the development of hyperinsulinaemic insulin resistance and abdominal fat deposits. Multiple case–control studies have reported hyperinsulinaemia to be a risk marker for breast cancer, most clearly in postmenopausal women [52]. Higher insulin levels tend to increase the bioavailability of insulin-like growth factor 1 by counteracting the production of the binding protein IGFBP3 [57]. A recent prospective study has confirmed a positive association between increased breast cancer risk and an elevated circulating level of insulin-like growth factor 1 [58], despite inconsistent results from previous case–control studies [52].

The expression of insulin and insulin-like growth factor 1 receptors has been shown in practically all primary breast cancers [59]. Both insulin-like growth factors 1 and 2 are expressed in the tumour stroma but their mitogenic effects are mediated mainly through the insulin-like growth factor 1 receptor in the epithelial component of breast cancer [60]. Synergism between oestrogen and insulin-like growth factor 1 has been shown to stimulate proliferative activity in human mammary cancer cells [61]. Insulin and insulin-like growth factor 1 binding is up to 10 times higher in primary breast cancers than in the adjacent normal breast tissue and is significantly higher in samples with overexpression of mutant p53 [62]. While interference with insulin-like growth factor receptor activation induces apoptosis in breast cancer cells *in vitro* [56], overexpression of mutated p53 may lead to increased expression of insulin and insulin-like growth factor 1 receptors [63].

Experimental evidence suggests that the expression of insulin-like growth factor receptors is associated with malignant transformation of breast epithelial cells [56], and increases the response in breast cancer cells to oestrogen [64]. A direct relationship has repeatedly been shown between ER and insulin-like growth factor receptor levels in human mammary cancer specimens [52]. Although an inverse relationship is observed between ER and epithelial growth factor receptor in breast cancer specimens, its significance is not clear [65].

Oestrogen may stimulate proliferation in breast cancer cells by an effect on cell signalling distal to insulin-like growth factor receptors [66] and a study of normal human breast tissue growing as a xenograft in nude mice has shown upregulation of insulin-like growth factor receptors by oestradiol [67]. Immunohistochemical localisation of insulin-like

growth factor receptors in benign and malignant breast tissue has shown its correlation with ER positivity in normal mammary epithelium, DCIS and invasive lobular carcinoma [68].

CONCLUSION

Cohort studies may help to elucidate the factors which influence progression of *in situ* carcinoma to invasive breast cancer. Considerable evidence suggests that at the menopause, many *in situ* lesions undergo spontaneous resolution presumably as a result of the falling oestrogen level. However, in genetically susceptible women, epigenetic factors associated with obesity and the Western lifestyle can contribute to the progression of a subgroup of premalignant lesions. The metabolic–endocrine changes which may be involved need to be correlated with the examination of biological markers in nipple aspirates, fine needle or core aspirates of breast tissue [9, 10].

Increased insulin-like growth factor 1 or insulin-like growth factor receptor expression may synergise with oestrogen in the late-stage promotion of mammary carcinogenesis in women approaching the menopause. Since premalignant lesions are nearer in time to these promoting factors, changes in their biomarkers may serve as surrogate endpoints long before epidemiological evidence of increased cancer incidence after the menopause.

Another application of such biomarkers may be to determine the effect of hormone replacement therapy on the risk of recurrence in women with a history of treated breast cancer. Large-scale randomised trials are in progress both in Europe and the U.S.A. and it may be feasible to incorporate observations on biomarker levels. Studies have reported an increased risk of *in situ* carcinoma from hormone replacement therapy [69, 70], and biological markers of premalignant changes may provide intermediate endpoints in future studies.

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