Mechanisms of Disease: prediction and prevention of breast cancer—cellular and molecular interactions

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SUMMARY

Breast cancer is the most prevalent female cancer in the world and its incidence is increasing, largely because of the Western lifestyle. There is a need, not only to predict women who will develop the disease, but also to apply drug and lifestyle measures in order to prevent the disease. Current risk prediction models are based on combinations of risk factors and have good predictive but low discriminatory power. New risk prediction methods might come from examination of single nucleotide polymorphisms in several genes or from an increased knowledge of the molecular and cellular biology of the breast, particularly with respect to aberrant gene expression and protein synthesis. These methods might also determine new targets for preventive agents and lifestyle change. Many potential preventive measures are available and some have been successful. New approaches are required, however, not only to prevent the disease but to devise methods for their assessment that do not require very large and expensive clinical trials.

KEYWORDS breast cancer, cellular interactions, prevention, risk, screening

REVIEW CRITERIA
The PubMed database was searched using Entrez for articles published up to 30 July 2005, including electronic early release publications. MEDLINE was searched for articles published up to August 2005 using OVID. The search terms included "breast cancer", "prediction", "prevention" and/or "risk", among others. The abstracts of retrieved citations were reviewed and prioritized by relative content. Full articles were obtained and references were checked for additional material when appropriate.

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INTRODUCTION

Although deaths from breast cancer are decreasing in many Western countries, the incidence of the disease is increasing, particularly in countries with a historically low incidence, such that breast cancer is now the world's most prevalent cancer.[1] The increase in incidence is almost certainly related to changes in dietary and reproductive patterns associated with Western lifestyles. There is increasing interest in prevention of the disease in order to save women the trauma of diagnosis and treatment. An overview of four major tamoxifen prevention trials showed a 38% reduction in breast cancer incidence in women who were at very high risk of the disease.[2] Despite this selection, it is estimated that over 50 women need to be treated to prevent a single case of breast cancer.[2]

Thus, along with a need to introduce new effective risk-reducing measures, there is also a need to improve the identification of women who are at increased risk of breast cancer. The two problems are interrelated because prediction of risk depends upon combining risk factors in order to give women their odds of developing breast cancer. Similarly, prevention partly depends upon reversing the cancer-inducing effects of risk factors such as hormones, reproductive factors and lifestyle factors (e.g. by inhibiting the estrogenic effects on breast epithelial cell proliferation) and reversing the negative effects of weight gain and sedentary lifestyle (Figure 1). Since all risk factors and preventive methods (Figure 2) must ultimately exert their effects upon breast tissue, it is important to determine how these effects might occur especially in light of cellular and molecular changes in the breast which, in turn, might give insight into new approaches for prediction and prevention. Here we outline the complexity of breast tissue and changes in its structure with age, and summarize current approaches to risk calculation and prevention. We also attempt to give some insight into what is currently known about molecular mechanisms in relation to the
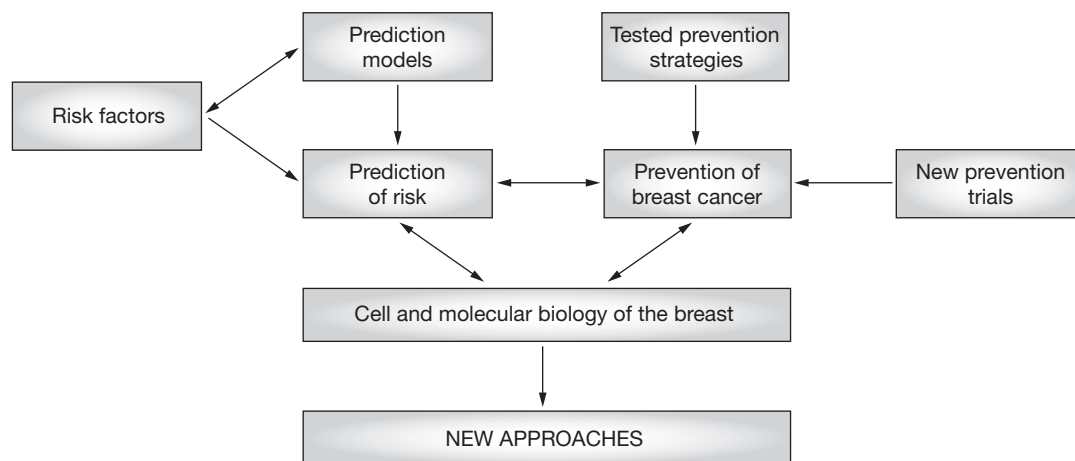
Figure 1 Prediction, prevention and biology of breast cancer. We propose that further study of the effects of risk factors and prevention strategies on the molecular biology of the breast is the key to new approaches.

cellular changes and interactions in the breast, disruption of which can lead to breast cancer, and summarize current preventative strategies and suggest new ones.

BREAST STRUCTURE: CHANGES WITH AGE

The breast arises from a specialized outgrowth of skin epithelium during the second trimester of pregnancy.[3] Estrogen receptor (ER) alpha gene expression begins in mammary epithelium at 30 weeks of intrauterine life and, in general, the numbers of epithelial cells that express the receptor increase with age.[4] Some breast secretory activity occurs at birth but the major development of the breast occurs during puberty, and cyclical proliferative activity in the epithelium continues until the menopause. Going and Moffat[5] have shown that as many as 15 ducts arise from the nipple, with each producing non-overlapping territories, which vary greatly in both area and ductal size (Figure 3A,B). In nulliparous women, breast lobules are small; they increase in size during pregnancy and then regress, though usually remain larger than nulliparous lobules (Figure 3C). The major tissue component of the breast is stroma. During early reproductive life breast tissue comprises approximately 20% epithelium, 20% fat and 60% connective tissue. These proportions change with age such that the amounts of epithelium and connective tissue decline[6] (Figure 3D) and the fat content increases. The density of breast tissue, as evaluated by mammograms, is thought to represent connective tissue and epithelium; this density also declines with age[7] (Figure 3E). Although increased breast density is clearly associated with increased risk, reduction in breast density with chronological age, and increase in density with weight loss, do not appear to correlate with an increased risk of cancer. It is of interest, and it might be important biologically, that changes in relative breast composition begin before the menopause. Although the epithelium volume declines with age, related to atrophy of lobules, many studies show that a large proportion of breasts in older women show features of premalignancy in some ducts and lobules. The increase in breast hyperplasia with age seen in two large postmortem studies is shown in Figure 3F.[8,9]

PREDICTION OF BREAST CANCER

Several methods of predicting risk of breast cancer in the clinic have been devised based on currently known risk factors.[10] Some depend on family history alone (e.g. the Claus and Ford models) and others depend upon hormonal and reproductive factors in addition to family history (e.g. the Gail and Tyrer–Cuzick models, Table 1, Figure 4). Outside of the clinics where most women have sufficiently strong family histories to have a high probability of harboring mutations in *BRCA1*, *BRCA2* and *TP53* genes, models that combine as many risk factors as possible are preferable. The Gail model accurately predicted the number of cancers in the Nurses Health Study,[11] but in our clinic, the Tyrer–Cuzick model, which depends on extent of family history and several endocrine factors, showed a better prediction than models using fewer risk factors (Table 1).[12] Although these models have good predictive power for the number of cancer cases likely to

be seen in a population, Rockhill *et al.*[11] point out that they have low discriminatory accuracy in that they cannot positively identify the particular woman who might develop breast cancer. It is estimated that for a genuine risk factor, differences in risk between high-risk and low-risk groups could need to be greater than a 100-fold in order to be used as a worthwhile screening test based upon the distribution of the relative risk within the whole population.[13] For example, alpha-fetoprotein is highly predictive for spina bifida, whereas serum cholesterol is not a good screening test for death from ischemic heart disease. Thus, a mutation in *BRCA1, BRCA2* or *TP53* is a useful test, but other breast cancer risk factors are relatively weak, alone or in combination, and might not represent good markers to test for risk. At present, studies are in progress to determine whether inclusion of additional factors to the models, such as mammographic density,[14] weight gain[15] and serum steroid hormone measurements,[16] will improve prediction.

Based on analysis of large numbers of families,[17] and on results of twin studies,[18] it has been suggested that most breast cancers will occur in a predictable minority of the population, and that efforts to determine methods of identifying this population are worthwhile. The concept of the 'at risk' breast is supported by the extensive and careful postmortem studies of Nielsen.[19] She found that newly-identified invasive and noninvasive breast cancers could be detected in the contralateral breast of 68% of women who died from breast cancer (this increased to 80% if contralateral metastases were included), whereas only 8% of breasts harbored malignancy in women dying of other causes. Two general approaches are being used to determine new markers of this apparent genetic risk. On the basis that single nucleotide polymorphisms (SNPs) might alter gene expression, many centers are attempting to determine a group of SNPs that might predict breast cancer.[20] An alternative approach is examining the expression of genes and proteins within the breast, which might predict risk, or perhaps focusing on other tissues (e.g. lymphocytes), since it would be very difficult to advocate breast biopsy on a population basis.

PREVENTION OF BREAST CANCER

To date, the most successful methods for preventing breast cancer have involved interference of estrogen activity either by blocking the ER (with agents such as tamoxifen and raloxifene) or by reducing estrogen concentrations by inhibiting the enzyme aromatase (with drugs such as anastrozole, letrozole and exemestane) (Figure 5). In four trials, tamoxifen prevented 38% of breast cancer cases[2] and raloxifene reduced breast cancer incidence in osteoporotic postmenopausal women by 59%.[21] The American/Canadian STAR (Study of Tamoxifen and Raloxifene) trial will soon report on the relative merits of the two drugs. Since both tamoxifen and raloxifene are effective preventative agents, the STAR trial is assessing the relative efficacy and tolerability of the drugs; it has completed trial entry of 19,747 subjects. Tamoxifen reduces contralateral breast cancer by 50% in premenopausal and postmenopausal women treated for an invasive cancer in the index breast. Aromatase inhibitors (AIs) reduced contralateral breast cancer by 50% compared with tamoxifen in randomized adjuvant endocrine therapy trials, indicating that AIs might reduce breast cancer overall by 70–80% compared with no treatment in postmenopausal women only.[22] Two clinical trials are investigating the possibility that AIs will prevent the majority of breast cancers: IBIS II (anastrozole versus placebo) and MA3 (exemestane versus placebo). In epidemiological studies, oophorectomy at 40 years of age reduces breast cancer risk by 50%; trials are in progress to determine whether temporary ovarian ablation with a gonadorelin analog for 2 years will have a similar effect.[23] In practice, tamoxifen is the only agent with FDA approval; it is not in widespread use, however, possibly because of the concern over side effects and its relative lack of specificity.

Other potential methods for breast cancer prevention are derived mainly from data from epidemiological studies. Adult weight gain can potentially double breast cancer risk;[15] we have recently shown in collaboration with the Iowa Women's Health Study group that weight reduction by at least 5% and weight loss maintenance in the premenopausal period reduced postmenopausal breast cancer risk by 39%, while weight loss in the postmenopausal period reduced risk by 25%.[24] As it is difficult to promote weight loss on a population basis there is interest in identifying energy restriction mimetic agents, which act by inhibiting glycolysis.[25,26] Most studies investigating the impact of exercise on breast cancer risk show a 20–30% reduction in breast cancer risk with
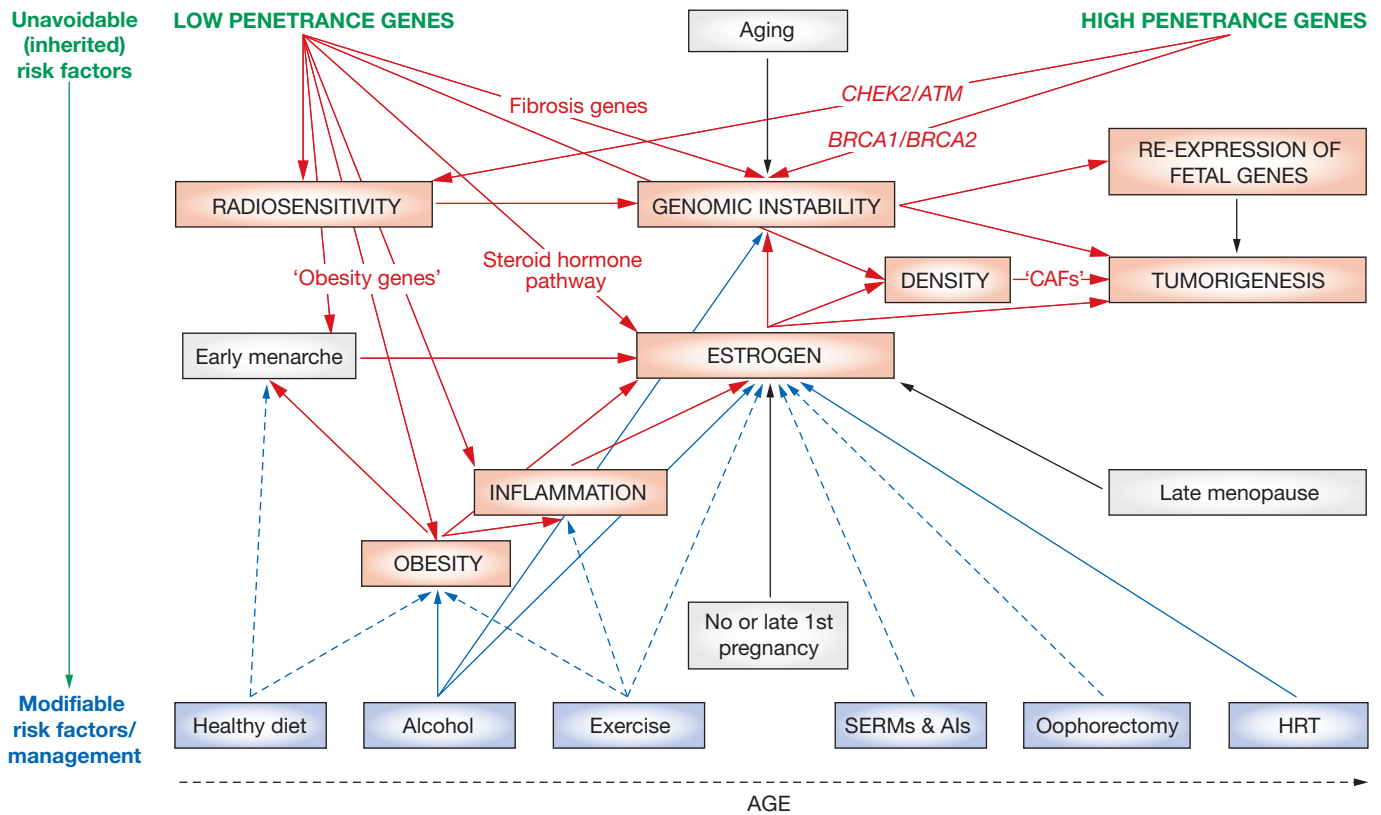
Figure 2 Overview of the many complex risk factors associated with breast cancer. The diagram summarizes the unavoidable (inherited) and modifiable risk factors that can ultimately lead to tumorigenesis. Genes/pathways/risk factors are shown in red; inherited or unmodifiable factors are shown in green; modifiable variables are shown in blue; life events are represented by gray boxes; increased/positive effects are denoted by solid arrows; and reduced/negative effects are denoted by dashed arrows. AIs, aromatase inhibitors; *ATM*, ataxia telangiectasia mutated; *BRCA*, breast cancer early onset; CAFs, cancer associated fibroblasts; *CHEK2*, CHK2 checkpoint homolog; HRT, hormone replacement therapy; SERMs, selective estrogen receptor modulators.

exercise, but many questions remain concerning the timing and extent of exercise required. Benefit might be gained from 1.25–2.5 hours of moderate exercise per week.[27] It is possible that both excess weight and lack of exercise mediate at least some of their risk through the inflammatory pathway. Some epidemiological studies,[28] but not all, suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) and statins[29] might reduce breast cancer risk. Clinical trials are required to substantiate these studies; however, in light of recent data showing an increased risk of colorectal cancer with the cyclo-oxygenase 2 (COX2) inhibitor, rofecoxib, this will be difficult to address.[30] One large randomized controlled trial in the US ($n = 39{,}876$), however, showed that 100 mg of aspirin on alternate days for a prolonged period is ineffective for breast cancer prevention.[31] A full discussion of potential agents

for prevention of breast cancer is beyond the scope of this article and the reader is referred to the recent reviews of Manson *et al.*[32] for discussion of the preventative value of natural products, and to Shen and Brown[33] for synthesized compounds; however, a summarized list of breast cancer preventative agents is given in Table 2.

CELLULAR AND MOLECULAR BIOLOGY OF THE BREAST

Understanding the biology of the breast is important for future risk prediction and reduction strategies. The normal and 'at risk' human breast is a difficult organ to study because of problems of access, and breast heterogeneity. Many data on the importance of cellular and molecular biology of the breast are derived from rodent or *in vitro* studies. Nevertheless these are important for our mechanistic understanding of the human breast

in situ. Genetic experiments on the rodent breast, especially during puberty, have highlighted the importance not only of the epithelium but also of interactions between these cells and other cell types within the breast (Figure 6), such as fibroblasts, adipocytes and macrophages.[34] For example, the breast does not develop when either parathyroid hormone-related peptide in the epithelium or its receptor on fibroblasts are knocked out. Insulin-like growth factor 1 from fibroblasts is absolutely required for breast development as is the recruitment of macrophages by colony stimulating factor 1 (CSF-1) secreted from the epithelium. Rodent mammary glands do not develop in the absence of white adipose tissue or leptin, which is secreted by this tissue.[35,36] Estrogen receptor signaling is essential for breast development as are a number of other signaling systems such as the Hedgehog (Hh) and Notch signaling pathways.[37] A critical role for Hh signaling in mediating stromal–epithelial interactions during ductal development has been demonstrated by the genetic analysis of two Hh signal transduction network genes, *Patched-1* (*Ptc-1*) and *Gli2*. Disruption of either gene leads to similar, yet distinct, defects in ductal morphogenesis—mainly ductal dysplasias similar to the hyperplasias of the human breast.[37,38] In other tissues, Notch signaling has been shown to have an important role in cell-fate determination, as well as in cell survival and proliferation.[39] The vertebrate *Notch4* gene is involved in normal mammary development. Transgenic mice expressing a constitutively active form of Notch4 fail to develop normal mammary glands and subsequently develop mammary tumors.[40] A role for Notch in human breast cancer growth has recently been described.[41] The importance of the stromal–epithelial interactions is demonstrated by experiments where the above factors—for example CSF-1—are overexpressed, resulting in malignancy. Interestingly, irradiation or carcinogen treatment of the cleared mammary fat pad results in neoplasia of reintroduced epithelial cells.[42,43]

It is clear that the majority of human breast neoplasms occur in the epithelium. Pathologists describe a continuum of epithelial abnormality from hyperplasia, atypical hyperplasia and carcinoma *in situ* to invasive neoplasm. Wellings *et al.*[44] described an atypical lobule thought to be the origin of breast cancer, which has since been renamed 'hyperplastic enlarged lobular unit' (HELU).[45] This structure is of
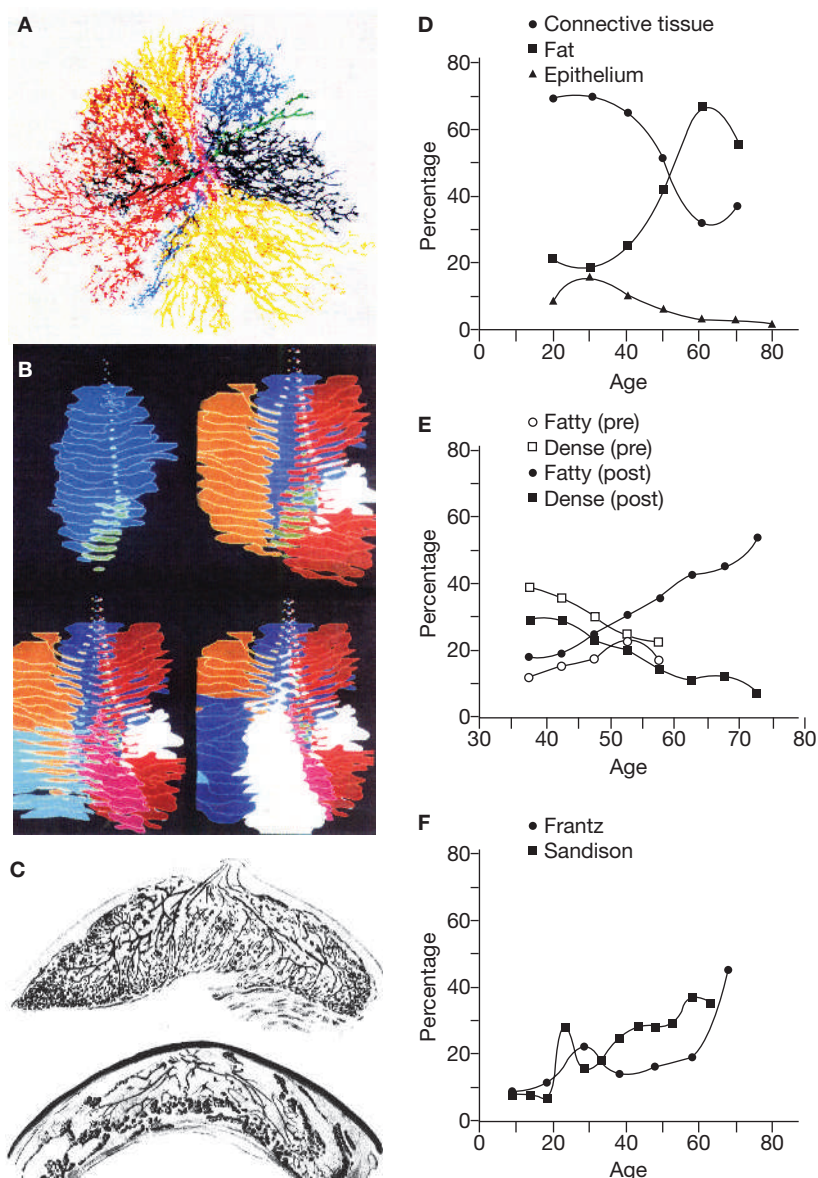

Figure 3 Anatomy and changes within the breast with age. (**A**) A face view of 15 ducts (I–XV) arising from the nipple with each producing non-overlapping territories that vary greatly in extent within the breast. Reproduced from Going JJ and Moffat DF (2004) Escaping from Flatland: clinical and biological aspects of human mammary duct anatomy in three dimensions. *J Pathol* **203:** 538–544 © (2004) Pathological Society of Great Britain and Ireland. Permission is granted by John Wiley and Sons on behalf of the the Pathological Society. (**B**) Four lateral views of duct areas with the nipple superior. The areas vary greatly in size and position. Reproduced with permission from Moffatt DF and Going JJ (1996) *J Clin Pathol* **49:** 48–52 © (1996) BMJ publishing group. (**C**) Upper diagram shows a cross section through the breast of a nulliparous 19-year-old and the lower diagram show a breast from a 27-year-old in the third month of pregnancy. Reproduced with permission from Dabelow A (1957) Die Milchdrüse. In *Handbuch der mikroskopischen Anatomie des Menschen*, III/3, 277–485 (Ed Bargman W) © (1957) Springer Science and Business Media. (**D**) Percentage of connective tissue, fat and epithelium with age, obtained using morphometry on 58 breasts. (**E**) Change in proportion of women with fatty and dense breasts with age using data from the breast cancer detection and demonstration project (*n* = 283,222 women).[7] Post, postmenopausal; pre, premenopausal. (**F**) Proportion of breasts containing areas of epithelial hyperplasia from two postmortem studies[8,9] (Frantz *et al.*, *n* = 225; Sandison, *n* = 800).

Table 1 Variables used in the Gail, Claus, Ford, Tyrer–Cuzick risk calculation models and the observed over expected ratios for each model in the Manchester population.[a]

	Gail	Claus	Ford	Tyrer–Cuzick
Prediction				
Follow-up study (observed/expected)	0.48	0.56	0.49	0.81
95% confidence interval	(0.54–0.90)	(0.59–0.99)	(0.52–0.80)	(0.85–1.41)
Personal information				
Age	Yes	Yes	Yes	Yes
Body mass index	No	No	No	Yes
Hormonal factors				
Menarche	Yes	No	No	Yes
First live birth	Yes	No	No	Yes
Menopause	No	No	No	Yes
HRT	No	No	No	Yes
Personal breast disease				
Breast biopsies	Yes	No	No	Yes
Atypical ductal hyperplasia	Yes	No	No	Yes
Lobular carcinoma *in situ*	No	No	No	Yes
Family History				
First degree relatives	Yes	Yes	Yes	Yes
Second degree relatives	No	Yes	Yes	Yes
Age of onset of breast cancer	No	Yes	Yes	Yes
Bilateral breast cancer	No	No	Yes	Yes
Ovarian cancer	No	No	Yes	Yes
Male breast cancer	No	No	Yes	No

[a]1,933 women who developed 52 cancers over a mean follow up period of 5.27 years.[12]

interest and, as seen with other hyperplasias, the proportion of ER-positive cells increases from about 20% in the normal breast to over 80% in hyperplasias and HELUs. Many of these ER-positive cells are highly proliferative, and rarely observed in the normal breast but common in invasive malignancy.[46] Some argue that breast cancers arise from ER-positive cells,[47] whereas others argue from experimental data on human cells that they arise from both ER-negative stem cells and ER-positive progenitor cells.[48,49] These considerations are not only of academic importance but are of future significance for prevention methods that might target stem cell self renewal pathways, for example, inhibition of the Hh pathway with agents such as cyclopamine or the Notch pathway with gamma secretase inhibitors.

Epithelial cells become genetically unstable during carcinogenesis, a phenomenon difficult to examine in the normal human breast *in situ*. Blood lymphocytes have therefore been used as surrogates. Studies demonstrate that women with, or at risk of, breast cancer, might have defective DNA repair and increased chromosomal radiosensitivity in both these cells.[50,51] Further investigation into these phenomena is needed in order to determine whether they will be efficient risk predictors. Blood samples are being collected from a large cohort of women in Norway (NOWAC study), which will constitute a large resource to study possible changes in lymphocytes that might reveal possible risk factors.[52]

Other predictors of risk might be early changes within the epithelium, if found to be widespread. Silencing of the tumor suppressor gene *p16INK4* associated with an increased expression of the stress-activated protein kinase p38 and COX2 have been described in approximately 30% of

women with histologically normal breasts.[53] Other findings of reduced apoptosis,[54] increased ER expression,[55] reduced integrin expression[56] and loss of heterozygosity[57] in 'normal' tissue might also indicate predictors of risk.

Fibroblasts are necessary for breast development and might play an important role in the adult mammary gland. Increased numbers of fibroblasts and collagen deposition have been demonstrated in mammographic dysplasia, and growth factor secretions stimulate tumor-cell growth.[58,59] Senescent fibroblasts secrete hepatocyte growth factor and other factors, which increases epithelial branching.[60] Human mammary fibroblasts are also vital to the 'uptake' of human epithelial cells in the fat pad of immune-deprived mice, a process that is enhanced if the fibroblasts are transduced with genes for hepatocyte growth factor and transforming growth factor-β (TGF-β).[61] Adipocytes secrete factors such as leptin and collagen VI, which stimulate epithelial cell growth.[62] Leptin also stimulates aromatase synthesis and ER transactivation,[63] and cytokines such as interleukin-6 and tumor-necrosis factor-α can stimulate aromatase activity indicating another method of stimulating epithelial cell growth by the production of estrogen. Leukocytes (e.g. macrophages) and inflammation might be critical to tumor development and progression (for review see Coussens and Werb[64]). An increased number of macrophages are detectable in experimental animals before the onset of neoplasia. It has been estimated that the percentage of macrophages in the adipose tissue of obese humans approaches 40% (compared with less than 10% in lean subjects) and it will be of interest to determine whether this also occurs in the breast.[65] The major cell types in the breast and their interactions mediated by secretory products are shown in Figure 6. The data to support this schema are derived from the studies summarized above. It is a major challenge to determine whether each cell is important in human mammary carcinogenesis, but such studies might produce new targets for prevention. Major questions are still unanswered. For example, how important are fibroblasts and their secretions in the risk associated with mammographic density, does calorie restriction act via adipocytes in the breast and do NSAIDs exert their effects directly on epithelial cells or via resident breast macrophages?
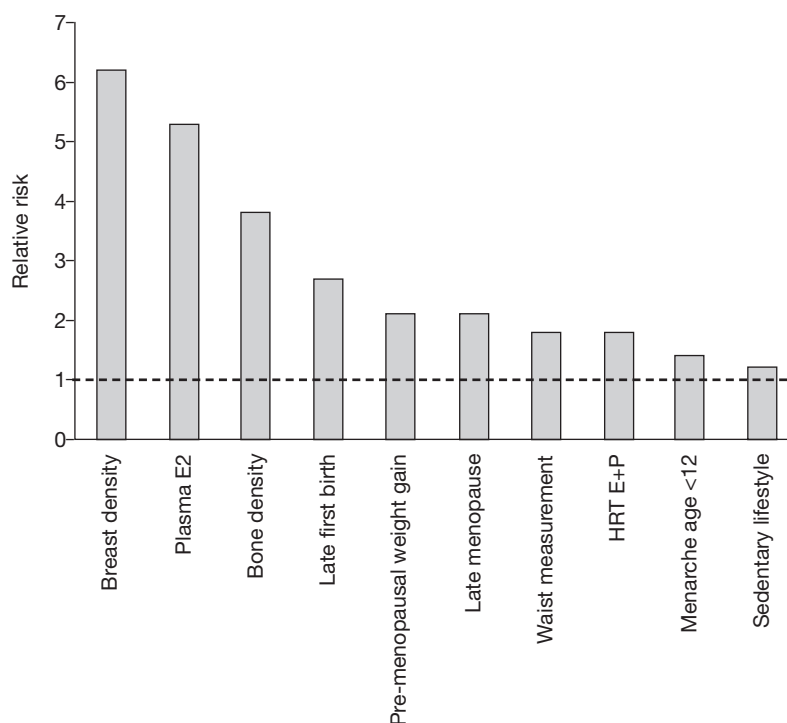

Figure 4 Estimated relative risk of breast cancer according to various endocrine and reproductive-related factors. E, estrogen; E2, estradiol; P, progesterone. Reproduced with permission from Larsen (2003) *Williams Textbook of Endocrinology* © (2003) Elsevier Inc.
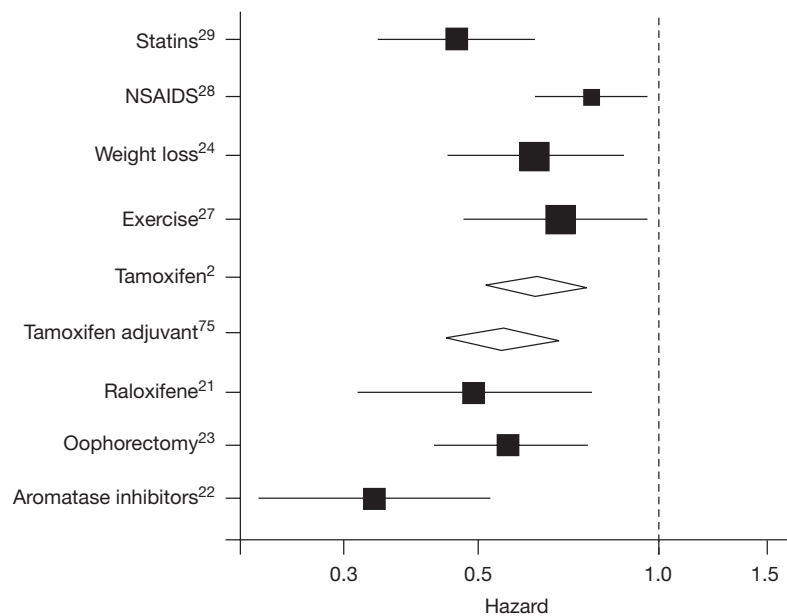

Figure 5 Forrest plot of interventions that reduce the risk of breast cancer. Comparison of the hazard ratios from selected epidemiological studies for statins, nonsteroidal anti-inflammatory drugs, weight loss and exercise. Tamoxifen and raloxifene data are from randomized trials. The oophorectomy data are from epidemiological observations and aromatase inhibitors from the ATAC trial. The plot is based on the assumption that tamoxifen reduces risk by 50% and anastrozole by a further 20–30%, since there was no placebo arm in the ATAC trial. Diamonds represent overviews with 95% confidence intervals; squares represent individual studies. Superscript numbers on Y-axis labels represent reference numbers.

Table 2 A summarized list of potential breast cancer prevention agents.[a]

Preventive agents	Class of agent/mechanism of action
Anti-hormones	
Tamoxifen, raloxifene, ospemifene, arzoxifene	SERM
Goserelin	Gonadotropin-releasing hormone analog
Anastrozole, letrozole, exemestane	Aromatase inhibitors
Phytoestrogens e.g. isoflavones	SERM-like
Downstream signaling modulation	
Retinoids (e.g. LGD1069, LG100268, fenretinide)	RAR/RXR agonists
Circumin	Inhibitor of AP1 and NFκB
Statins	Inhibitor of NFκB
Parthenolide	Inhibitor of NFκB
Selenium	ERα signaling disruption
2-deoxyglucose	Downregulation of AMPK/Akt signaling pathways
Thiazolidinediones	PPARγ pathway agonism
Non-steroidal anti-inflammatory drugs	COX2 inhibitor
Huanglian (Chinese herb)	Interferon-β upregulation
C75	Fatty acid synthase inhibitor
Resveratrol	SIRT1 activator, multiple other targets
Green/black tea (polyphenols)	Multiple targets
Indole-3-carbinol	Multiple targets

[a]For full details see Manson et al.[32] for discussion of the preventative value of natural products and Shen and Brown[33] for synthesized compounds.
Akt, v-akt murine thymoma viral oncogene homolog 1; AMPK, adenosine monophosphate activated protein kinase; AP1, v-jun sarcoma virus 17 oncogene homolog (avian); COX2, cyclo-oxygenase 2; ERα, estrogen receptor alpha; NFκB, nuclear factor of kappa light chain gene enhancer in B-cells; PPARγ, peroxisome proliferator-activated receptor gamma; RAR, retinoic acid receptor; RXR, retinoid X receptor; SERM, selective estrogen receptor modulators; SIRT1, sirtuin (silent mating type information regulation 2 homolog) 1.

GENE EXPRESSION WITHIN THE BREAST

Various platforms and approaches have begun to show how gene expression profiles might differ under various circumstances within the breast, which could give an indication of risk. Young age of first birth is associated with reduced breast cancer risk; a gene expression comparison of the nulliparous versus the parous breast[66] showed that differentiation markers were expressed in the parous breast, and growth and extracellular matrix genes in the nulliparous breast. In HELUs, Allred and colleagues showed G-protein over-expression and increased retinoic acid pathway signaling compared with normal breast tissue.[47] Extensive microarray experiments on human invasive tumors, ductal carcinoma *in situ* and stromal components especially myofibroblasts have shown expression profiles unique to types of tumor and tumor stroma.[67,68] CXCL12 and CXCL14 chemokines, overexpressed in tumor myoepithelial cells and myofibroblasts, respectively, bind to receptors on epithelial cells and enhance their proliferation, migration and invasion. Thus, chemokines might play a role in breast tumorigenesis by acting as paracrine factors.[68] If some of these genes were overexpressed in some normal breast tissue they might constitute risk markers. Interestingly the 'wound profile' generated by Chang and colleagues[69] on the transcriptional response of normal fibroblasts to serum is a poor prognostic factor in human breast tumors; it was previously thought that the molecular program of normal wound healing might play an important role in cancer metastasis. Senescent fibroblasts tend to overexpress wound repair genes[70] and preadipocytes over-express genes for extracellular matrix components.[71] In obese subjects, inflammatory gene expression in abdominal subcutaneous fat was altered; there was increased expression of

proinflammatory genes and decreased expression of anti-inflammatory genes in abdominal subcutaneous fat of obese compared with non-obese subjects, which reverted to normal with one month period of diet-induced weight reduction.[71] This is an important study since it demonstrates that extensive information based upon gene expression arrays might be obtained by biopsy before and after short-term human lifestyle intervention.

MEDIATORS OF PREVENTION

Greater understanding of more general mediators of breast cancer risk might allow the development of new novel therapies for breast cancer prevention. Risk factors such as obesity and inflammation are associated with changes in intracellular signaling pathways, which could be the targets of preventive agents and lifestyle change. Interactions between these pathways and response to agents might not only give an indication of the mechanisms of action of current agents but could lead to the development of new agents. Nuclear factor kappa B (NFκB) has been shown to be a central mediator of the development of malignancy in epithelia and also of tumor promotion by macrophage infiltration, since inhibition of NFκB activity in either cell type is associated with reduced tumor formation, decreased inflammation and increased apoptosis.[72] Indeed NFκB has been called a 'major culprit' in tumor development,[73] and its activity is reduced by dietary energy restriction by reduction of AMP-related kinase and Akt/protein kinase B, and can also be reduced by increased SIRT1 (silent mating type information regulation 2 homolog) activity. SIRT1 is the human homologue of SIR2, a histone deacetylase that is associated with longevity in *Caenorhabditis elegans* and *Drosophila*.[74] Accordingly, as resveratrol is known to increase SIRT1, this might be one of the mechanisms of resveratrol's highly favorable preventive effects in experimental systems.[75] NFκB overexpression inhibits the activity of the ER,[76] and parthenolide, an NFκB inhibitor, can reverse subsequent tamoxifen and fulvestrant resistance in mammary tumor cell lines.[77,78] NFκB also upregulates COX2 via mechanisms involving retinoids,[79] which leads to increased prostaglandin synthesis, aromatase activity and cell proliferation. In Figure 7 we summarize some of the interactions, which might occur between these pathways, and how they might be inhibited by known chemoprevention agents and lifestyle
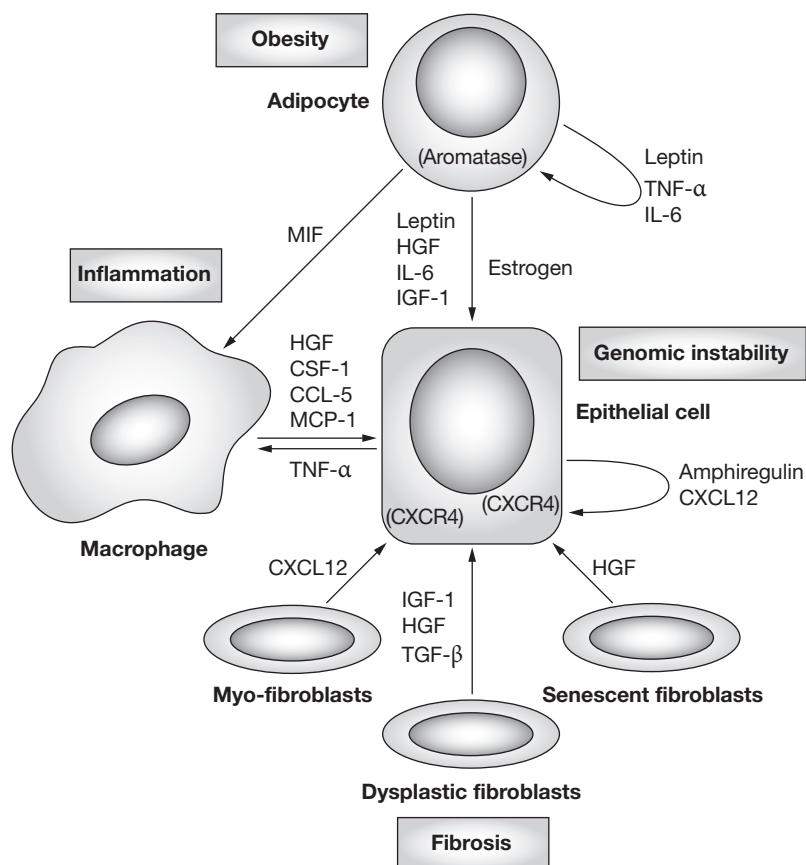

Figure 6 Cellular interactions between different cell types within human breast tissue. Boxes represent the risk factors associated with specific breast cell types. The arrows represent signaling between the cell types.
CCL5, chemokine ligand 5; CSF-1, colony-stimulating factor; CXCL12, chemokine ligand 12 (stromal cell-derived factor-1); CXCR4, chemokine receptor 4; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; MCP-1, macrophage chemoattractant protein; MIF, macrophage migration inhibitory factor (glycosylation-inhibiting factor); TGF-β1, transforming growth factor-beta 1; TNF-α, tumor-necrosis factor-alpha.

change. It seems likely that in the future, a small number of preventive agents in combination, along with lifestyle changes, could be the answer to the prevention of breast cancer.

If the cellular interactions shown in Figure 6 are found to be important, it could be that we need to consider inhibiting the activity of more than one cell type: for example; energy restriction or energy restriction mimetic agents for adipocytes, NSAIDs for macrophages, selective estrogen receptor modulators (SERMs) or AIs for ER-positive epithelial cells and growth factor pathway inhibitors for ER-negative epithelial cells. Alternatively, if NFκB is a 'major culprit' in tumor development, several pathways of inhibition might be more
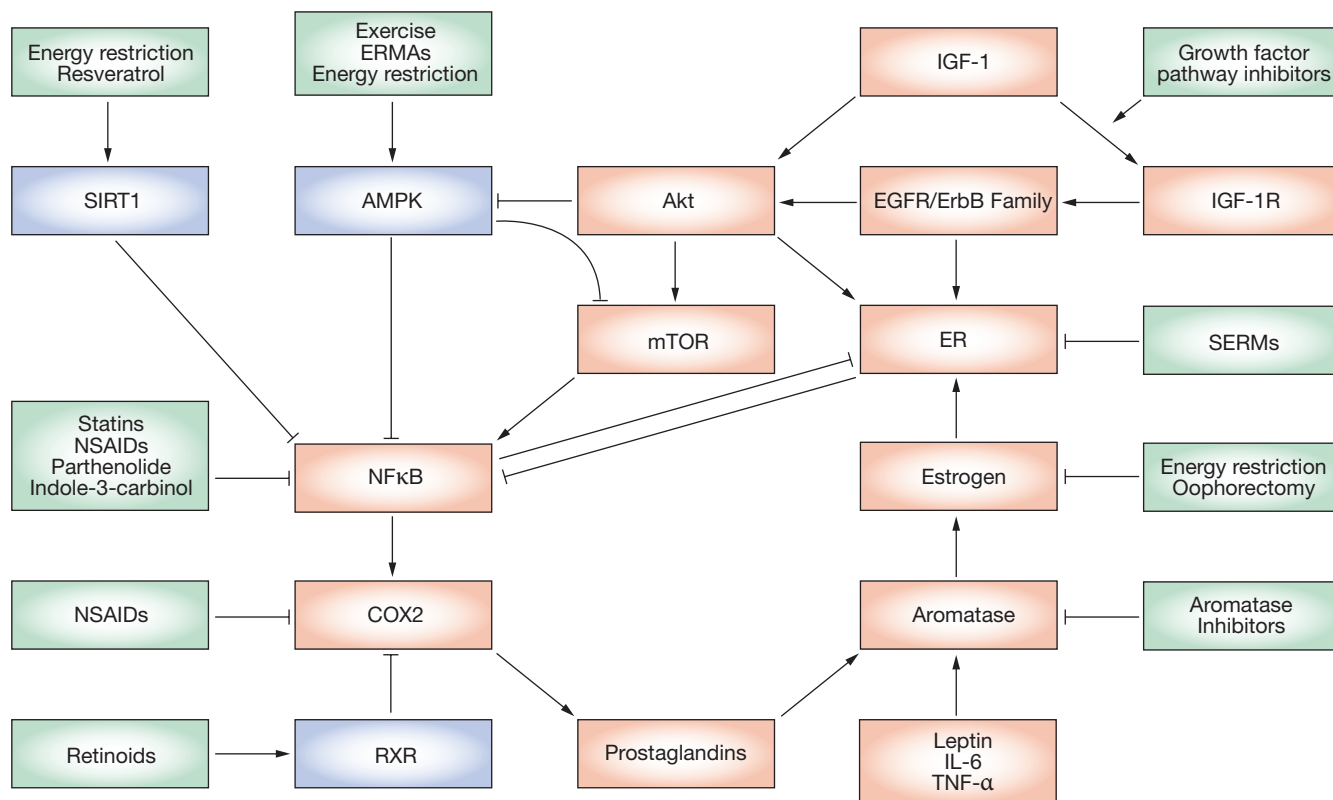
Figure 7 Molecular targets of potential preventive strategies. Factors promoting the development of cancer are shown in red; factors that inhibit carcinogenesis are shown in blue. Positive or inhibitory effects are shown by arrows. Cancer prevention strategies are shown in green.

Akt, v-akt murine thymoma viral oncogene homolog 1; AMPK, adenosine monophosphate activated protein kinase; COX2, cyclo-oxygenase-2; EGFR, epidermal growth factor receptor; ER, estrogen receptor alpha; ERMAs, energy restriction mimetic agents; IL-6, interleukin-6; IGF-1, insulin-like growth factor 1; IGF-1R, insulin-like growth factor 1 receptor; mTOR, mammalian target of rapamycin; NFκB, nuclear factor of kappa light chain gene enhancer in B-cells; NSAIDs, nonsteroidal anti-inflammatory drugs; SERMs, selective estrogen receptor modulators; RXR, retinoid X receptor; SIRT1, sirtuin (silent mating type information regulation 2 homolog) 1; TNF-α, tumor-necrosis factor-alpha.

effective than a single one, so that energy restriction, and preventative agents such as SERMs or AIs and parthenolide or statins together might be more effective than either alone. The likely site of action of these interventions is shown in Figure 7. The difficulty lies in testing such approaches. Needle or core biopsy before and after such interventions is clearly an important approach and is being tested in several centers.[80,81]

CONCLUSION

The human breast is a highly complex, difficult organ to investigate; it changes in composition with time at different rates in different people. Current risk prediction methods depend upon a compendium of risk factors organized into risk prediction models, which have good predictive but low discriminatory value. Risk estimation could be improved by incorporating additional factors into risk prediction models. This might be achieved by the identification of new predictors based on SNP array or increased knowledge of the molecular and cellular biology of the breast, in part from gene array and examination of the breast proteome as outlined above. These factors might also be new targets for prevention. Several drug and lifestyle targets have already been delineated and require testing in clinical trials. Nonetheless, new methods to determine the effect of new approaches on the breast—without or before embarking on large and expensive clinical trials—are required.

References
1   Parkin DM *et al.* (2005) Global cancer statistics, 2002. *CA Cancer J Clin* **55:** 74–108
2   Cuzick J *et al.* (2003) Overview of the main outcomes in breast-cancer prevention trials. *Lancet* **361:** 296–300
3   Howard BA and Gusterson BA (2000) Human breast development. *J Mammary Gland Biol Neoplasia* **5:** 119–137

4   Keeling JW *et al.* (2000) Oestrogen receptor alpha in female fetal, infant, and child mammary tissue. *J Pathol* **191:** 449–451

5   Going JJ and Moffat DF (2004) Escaping from Flatland: clinical and biological aspects of human mammary duct anatomy in three dimensions. *J Pathol* **203:** 538–544

6   Hutson SW *et al.* (1985) Morphometric studies of age related changes in normal human breast and their significance for evolution of mammary cancer. *J Clin Pathol* **38:** 281–287

7   Brisson J *et al.* (1988) Mammographic parenchymal features and breast cancer in the breast cancer detection demonstration project. *J Natl Cancer Inst* **80:** 1534–1540

8   Frantz VK *et al.* (1951) Incidence of chronic cystic disease in so-called "normal breasts"; a study based on 225 postmortem examinations. *Cancer* **4:** 762–783

9   Sandison AT (1962) An autopsy study of the adult human breast: with special reference to proliferative epithelial changes of importance in the pathology of the breast. *Natl Cancer Inst Monogr* **4:** 1–145

10   Freedman AN *et al.* (2005) Cancer risk prediction models: a workshop on development, evaluation, and application. *J Natl Cancer Inst* **97:** 715–723

11   Rockhill B *et al.* (2001) Validation of the Gail *et al.* model of breast cancer risk prediction and implications for chemoprevention. *J Natl Cancer Inst* **93:** 358–366

12   Amir E *et al.* (2003) Evaluation of breast cancer risk assessment packages in the family history evaluation and screening programme. *J Med Genet* **40:** 807–814

13   Wald NJ *et al.* (1999) When can a risk factor be used as a worthwhile screening test? *BMJ* **319:** 1562–1565

14   Boyd NF *et al.* (1998) Mammographic densities and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **7:** 1133–1144

15   Huang Z *et al.* (1997) Dual effects of weight and weight gain on breast cancer risk. *JAMA* **278:** 1407–1411

16   Key TJ *et al.* (2003) Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *J Natl Cancer Inst* **95:** 1218–1226

17   Pharoah PD *et al.* (2002) Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet* **31:** 33–36

18   Peto J and Mack TM (2000) High constant incidence in twins and other relatives of women with breast cancer. *Nat Genet* **26:** 411–414

19   Nielsen M (1989) Autopsy studies of the occurrence of cancerous, atypical and benign epithelial lesions in the female breast. *APMIS* **10 (Suppl):** 1–56

20   Pharoah PD *et al.* (2004) Association studies for finding cancer-susceptibility genetic variants. *Nat Rev Cancer* **4:** 850–860

21   Martino S *et al.* (2004) Continuing outcomes relevant to Evista: breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene. *J Natl Cancer Inst* **96:** 1751–1761

22   Howell A *et al.* (2005) Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet* **365:** 60–62

23   Olson JE *et al.* (2004) Bilateral oophorectomy and breast cancer risk reduction among women with a family history. *Cancer Detect Prev* **28:** 357–360

24   Harvie M *et al.* (2005) Association of gain and loss of weight before and after menopause with risk of postmenopausal breast cancer in the Iowa women's health study. *Cancer Epidemiol Biomarkers Prev* **14:** 656–661

25   Zhu Z *et al.* (2005) 2-deoxyglucose as an energy restriction mimetic agent: effects on mammary carcinogenesis and on mammary tumor cell growth *in vitro*. *Cancer Res* **65:** 7023–7030

26   Ingram DK *et al.* (2004) Development of calorie restriction mimetics as a prolongevity strategy. *Ann NY Acad Sci* **1019:** 412–423

27   McTiernan A *et al.* (2003) Recreational physical activity and the risk of breast cancer in postmenopausal women: the Women's Health Initiative Cohort Study. *JAMA* **290:** 1331–1336

28   Zhang Y *et al.* (2005) Use of nonsteroidal antiinflammatory drugs and risk of breast cancer: the Case-Control Surveillance Study revisited. *Am J Epidemiol* **162:** 165–170

29   Kochhar R *et al.* (2005) Statins to reduce breast cancer risk: a case control study in US female veterans. In *Proceedings of the American Society of Clinical Oncology Annual Meeting: 2005 May 13–17; Florida.* ASCO

30   Bresalier RS *et al.* (2005) Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med* **352:** 1092–1102

31   Cook NR *et al.* (2005) Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. *JAMA* **294:** 47–55

32   Manson MM *et al.* (2005) Innovative agents in cancer prevention. *Recent Results Cancer Res* **166:** 257–275

33   Shen Q and Brown PH (2003) Novel agents for the prevention of breast cancer: targeting transcription factors and signal transduction pathways. *J Mammary Gland Biol Neoplasia* **8:** 45–73

34   Wiseman BS and Werb Z (2002) Stromal effects on mammary gland development and breast cancer. *Science* **296:** 1046–1049

35   Couldrey C *et al.* (2002) Adipose tissue: a vital *in vivo* role in mammary gland development but not differentiation. *Dev Dyn* **223:** 459–468

36   Hu X *et al.* (2002) Leptin—a growth factor in normal and malignant breast cells and for normal mammary gland development. *J Natl Cancer Inst* **94:** 1704–1711

37   Lewis MT *et al.* (1999) Defects in mouse mammary gland development caused by conditional haploinsufficiency of *Patched-1*. *Development* **126:** 5181–5193

38   Lewis MT (2001) Hedgehog signaling in mouse mammary gland development and neoplasia. *J Mammary Gland Biol Neoplasia* **6:** 53–66

39   Lai EC (2004) Notch signaling: control of cell communication and cell fate. *Development* **131:** 965–973

40   Gallahan D and Callahan R (1997) The mouse mammary tumor associated gene *INT3* is a unique member of the *NOTCH* gene family (*NOTCH4*). *Oncogene* **14:** 1883–1890

41   Pece S *et al.* (2004) Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. *J Cell Biol* **167:** 215–221

42   Barcellos-Hoff MH and Ravani SA (2000) Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. *Cancer Res* **60:** 1254–1260

43   Maffini MV *et al.* (2004) The stroma as a crucial target in rat mammary gland carcinogenesis. *J Cell Sci* **117:** 1495–1502

44   Wellings SR *et al.* (1975) An atlas of subgross pathology of the human breast with special reference to possible precancerous lesions. *J Natl Cancer Inst* **55:** 231–273

45   Lee S *et al.* (2005) Biological features and xenograft models of a very early human premalignant breast lesion. *Breast Cancer Res* **7(Suppl 2):** S17

46   Clarke RB *et al.* (1997) Dissociation between steroid receptor expression and cell proliferation in the human breast. *Cancer Res* **57:** 4987–4991

47   Allred DC *et al.* (2004) The origins of estrogen receptor alpha-positive and estrogen receptor alpha-negative human breast cancer. *Breast Cancer Res* **6:** 240–245

48 Clarke RB *et al.* (2005) A putative human breast stem cell population is enriched for steroid receptor-positive cells. *Dev Biol* **277:** 443–456

49 Dontu G *et al.* (2004) Breast cancer, stem/progenitor cells and the estrogen receptor. *Trends Endocrinol Metab* **15:** 193–197

50 Kennedy DO *et al.* (2005) DNA repair capacity of lymphoblastoid cell lines from sisters discordant for breast cancer. *J Natl Cancer Inst* **97:** 127–132

51 Scott D (2004) Chromosomal radiosensitivity and low penetrance predisposition to cancer. *Cytogenet Genome Res* **104:** 365–370

52 Borresen-Dale AL and Sharma P (2005) Expression profiling of peripheral blood cells for early detection. *Breast Cancer Res* **7 (Suppl 2):** S8

53 Gauthier ML *et al.* (2005) p38 regulates cyclooxygenase-2 in human mammary epithelial cells and is activated in premalignant tissue. *Cancer Res* **65:** 1792–1799

54 Allan DJ *et al.* (1992) Reduction in apoptosis relative to mitosis in histologically normal epithelium accompanies fibrocystic change and carcinoma of the premenopausal human breast. *J Pathol* **167:** 25–32

55 Khan SA *et al.* (1999) The normal breast epithelium of women with breast cancer displays an aberrant response to estradiol. *Cancer Epidemiol Biomarkers Prev* **8:** 867–872

56 Hassan HI and Walker RA (2001) Altered expression of epidermal growth factor receptor in non-involved tissue of cancer-containing breasts. *Breast* **10:** 318–324

57 Deng G *et al.* (1996) Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science* **274:** 2057–2059

58 Guo YP *et al.* (2001) Growth factors and stromal matrix proteins associated with mammographic densities. *Cancer Epidemiol Biomarkers Prev* **10:** 243–248

59 Tlsty TD *et al.* (2005) Epigenetic and genetic changes control tumorigenic phenotypes and occur *in vivo* in human mammary epithelia. In *Proceedings of Keystone Symposium: The Role of Microenvironment in Tumor Induction and Progression: 2005 February 5–10; Banff*. Silverthorne: Keystone Symposia

60 Parrinello S *et al.* (2005) Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. *J Cell Sci* **118:** 485–496

61 Kuperwasser C *et al.* (2004) Reconstruction of functionally normal and malignant human breast tissues in mice. *Proc Natl Acad Sci USA* **101:** 4966–4971

62 Iyengar P *et al.* (2005) Adipocyte-derived collagen VI affects early mammary tumor progression *in vivo*, demonstrating a critical interaction in the tumor/stroma microenvironment. *J Clin Invest* **115:** 1163–1176

63 Catalano S *et al.* (2004) Leptin induces, via ERK1/ERK2 signal, functional activation of estrogen receptor alpha in MCF-7 cells. *J Biol Chem* **279:** 19908–19915

64 Coussens LM and Werb Z (2002) Inflammation and cancer. *Nature* **420:** 860–867

65 Weisberg SP *et al.* (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* **112:** 1796–1808

66 Verlinden I *et al.* (2005) Parity-induced changes in global gene expression in the human mammary gland. *Eur J Cancer Prev* **14:** 129–137

67 Perou CM *et al.* (2000) Molecular portraits of human breast tumours. *Nature* **406:** 747–752

68 Allinen M *et al.* (2004) Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell* **6:** 17–32

69 Chang HY *et al.* (2005) Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. *Proc Natl Acad Sci USA* **102:** 3738–3743

70 Shelton DN *et al.* (1999) Microarray analysis of replicative senescence. *Curr Biol* **9:** 939–945

71 Clement K *et al.* (2004) Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *FASEB J* **18:** 1657–1669

72 Greten FR *et al.* (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118:** 285–296

73 Karin M *et al.* (2002) NF-κB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* **2:** 301–310

74 Luo Z et al. (2005) AMPK, the metabolic syndrome and cancer. *Trends Pharmacol Sci* **26:** 69–76

75 Lamming DW *et al.* (2004) Small molecules that regulate lifespan: evidence for xenohormesis. *Mol Microbiol* **53:** 1003–1009

76 Kalaitzidis D and Gilmore TD (2005) Transcription factor cross-talk: the estrogen receptor and NF-kappaB. *Trends Endocrinol Metab* **16:** 46–52

77 deGraffenried LA *et al.* (2004) NF-kappa B inhibition markedly enhances sensitivity of resistant breast cancer tumor cells to tamoxifen. *Ann Oncol* **15:** 885–890

78 Riggins RB *et al.* (2005) The nuclear factor kappa B inhibitor parthenolide restores ICI 182,780 (Faslodex; fulvestrant)-induced apoptosis in antiestrogen-resistant breast cancer cells. *Mol Cancer Ther* **4:** 33–41

79 Kong G *et al.* (2005) The retinoid X receptor-selective retinoid, LGD1069, down-regulates cyclooxygenase-2 expression in human breast cells through transcription factor crosstalk: implications for molecular-based chemoprevention. *Cancer Res* **65:** 3462–3469

80 Harper-Wynne C *et al.* (2002) Effects of the aromatase inhibitor letrozole on normal breast epithelial cell proliferation and metabolic indices in postmenopausal women: a pilot study for breast cancer prevention. *Cancer Epidemiol Biomarkers Prev* **11:** 614–621

81 Fabian CJ *et al.* (2005) Breast-tissue sampling for risk assessment and prevention. *Endocr Relat Cancer* **12:** 185–213