Available online at www.sciencedirect.com
SCIENCE DIRECT®
Maturitas 49 (2004) 34–43
MATURITAS
THE EUROPEAN MENOPAUSE JOURNAL
www.elsevier.com/locate/maturitas

Oncogenic pathways in hereditary and sporadic breast cancer

P. Kenemans*, R.A. Verstraeten, R.H.M. Verheijen

Department of Obstetrics and Gynaecology, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands

Received 8 April 2004; received in revised form 10 June 2004; accepted 10 June 2004

Abstract

Cancer is a genetic disease. Breast cancer tumorigenesis can be described as a multi-step process in which each step is thought to correlate with one or more distinct mutations in major regulatory genes. The question addressed is how far a multi-step progression model for sporadic breast cancer would differ from that for hereditary breast cancer.

Hereditary breast cancer is characterized by an inherited susceptibility to breast cancer on basis of an identified germline mutation in one allele of a high penetrance susceptibility gene (such as BRCA1, BRCA2, CHEK 2, TP53 or PTEN). Inactivation of the second allele of these tumour suppressor genes would be an early event in this oncogenic pathway (Knudson's "two-hit" model).

Sporadic breast cancers result from a serial stepwise accumulation of acquired and uncorrected mutations in somatic genes, without any germline mutation playing a role. Mutational activation of oncogenes, often coupled with non-mutational inactivation of tumour suppressor genes, is probably an early event in sporadic tumours, followed by more, independent mutations in at least four or five other genes, the chronological order of which is likely less important. Oncogenes that have been reported to play an early role in sporadic breast cancer are MYC, CCND1 (Cyclin D1) and ERBB2 (HER2/neu). In sporadic breast cancer, mutational inactivation of BRCA1/2 is rare, as inactivation requires both gene copies to be mutated or totally deleted. However, non-mutational functional suppression could result from various mechanisms, such as hypermethylation of the BRCA1 promoter or binding of BRCA2 by EMSY.

In sporadic breast tumorigenesis, at least three different pathway-specific mechanisms of tumour progression are recognizable, with breast carcinogenesis being different in ductal versus lobular carcinoma, and in well differentiated versus poorly differentiated ductal cancers.

Thus, different breast cancer pathways emerge early in the process of carcinogenesis, ultimately leading to clinically different tumour types. As mutations acquired early during tumorigenesis will be present in all later stages, large-scale gene expression profiling using DNA microarray analysis techniques can help to classify breast cancers into clinically relevant subtypes.

Keywords: Tumorigenesis; Breast cancer; Hereditary

1. Introduction

Breast cancer is a genetic disease. At the moment breast cancer is diagnosed clinically, mutations can be demonstrated in at least four to six major regulatory

* Corresponding author.
E-mail address: Kenemans@vumc.nl (P. Kenemans).

0378-5122/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.maturitas.2004.06.005

genes, located on various chromosomes present in the nucleus of the breast cancer cell. These genes play a role in maintaining the physiological balance between proliferation, apoptosis and differentiation. Other genes regulate expression of steroid receptors, cell adhesion molecules and angiogenic factors, and of various other proteins important for invasion and the establishment of metastases.

It has been proposed that the process of breast cancer tumorigenesis is best described by a multi-step progression model [1], in which the normal breast epithelium evolves via hyperplasia and carcinoma in situ into an invasive cancer, which eventually can disseminate via lymph and blood vascular systems to form metastases. Each of these steps is thought to correlate with one or more distinct mutations in regulatory genes. The question can be raised in how far the multi-step progression model proposed for sporadic breast cancer differs from that proposed for hereditary breast cancer.

Hereditary breast cancer constitutes approximately half of all cases of familial breast cancer, here defined as women with two or more family members diagnosed with breast cancer before the age of 60 years. Hereditary breast cancer is found in women with evidence of inherited susceptibility to breast cancer on basis of an identified germline mutation. The major high penetrance genes in which mutations increase susceptibility to breast cancer are the breast cancer susceptibility gene 1 (BRCA1) and the breast cancer susceptibility gene 2 (BRCA2). Mutations in these genes account together for 2–3% of all breast cancers and around 30–40% of all familial breast cancers [2].

In addition to BRCA1 and 2, various other genes have been found to be mutated in familial breast cancer. Mutations in the cell cycle checkpoint kinase gene (CHEK 2) account for about 5% of familial cancer cases. Mutations in TP53 (causing the Li-Fraumeni Syndrome) and those in PTEN (Cowden's disease) are responsible for no more than 1% of all familial breast cancer cases. Additional high penetrance genes that increase susceptibility to breast cancer (but not to ovarian cancer) are likely to exist [2].

Predisposition to breast cancer has also been related to a variety of genetic polymorphisms in genes involved in metabolism of steroid hormones (e.g. CYP17 and CYP19) and of carcinogens (e.g. CYP1A1, NAT1 and NAT2) [3].

By far, the majority of breast cancers are so-called sporadic cancers that result from the accumulation of acquired and uncorrected genetic alterations in somatic genes, without any germline mutation playing a role. Risk factors for sporadic breast cancer are often hormonal in nature [4].

2. The multi-step progression model of breast cancer

Breast development begins in the embryonic period. Ductal morphogenesis starts from a bud-like structure with branching, elongation and then canalization. Basal cells, expressing both smooth muscle actin, as well as high molecular weight cytokeratins appear at the end of the second trimester. In the adult breast two major cell types can be distinguished: the myoepithelial cell and the luminal secretory cell. Clinically and histopathologically, various steps can be identified during progression to malignancy [5].

Ductal hyperplasia, characterized by proliferation of unevenly distributed epithelial cells with nuclei of varying shape and chromatin pattern, is often a first sign of pathology. The cells have relatively little cytoplasm and no clear cell borders. Cytologically the cells are benign. The transition from hyperplasia to atypical hyperplasia is clinically associated with an increased risk of breast cancer. The next step is development of carcinoma in situ, either ductal or lobular, which is defined as a proliferation of cells with cytological characteristics of malignancy, but without stromal invasion across the basement membrane.

As cells detach from the basement membrane and invade the stroma, the tumour becomes invasive. Through dissemination via blood and lymph vessels, invasive cells can give rise to metastases, either to locoregional lymph nodes or to distant organs. The majority of invasive carcinomas are ductal (85–95%). Infiltrating lobular carcinoma constitutes approximately 10% of all breast cancers.

In the classic model of multi-stage tumour development, a normal epithelial cell develops into a premalignant atypical cell, and after clonal expansion becomes a premalignant lesion, a carcinoma in situ (step 1), then after some time, such lesion may become invasive (step 2), then disseminates and, after evading the immune system, forms metastases (step 3).

At each step, an important genetic event is assumed to occur that gives the cell new properties with a resulting clonal selective advantage for that cell. These genetic events range from small point mutations, via chromosomal deletions, translocations and amplifications to large-scale changes as whole chromosome losses or duplications. The result of these alterations could be modification of gene expression or functional alteration of gene products that are relevant for tumour progression.

Mutational activation of oncogenes coupled with inactivation of tumour suppressor genes are probably early events in this multi-step process. Subsequently, more independent mutations occur in at least four or five other genes, the chronological order of these events possibly being less important.

Alterations in genes involved in regulation of mitosis and DNA repair mechanisms could lead to a situation of so-called genomic instability, in which the integrity of the genome is at risk. Aberrations that activate or inactivate various other genes can result, leading to further progression to a malignant phenotype [1,6]. Genomic instability might result from genetic events in single nucleotides, in micro-satellites (small stretches of DNA), in whole genes or in complete chromosomes or parts of it. Micro-satellite instability can result from both germline mutations, as well as from somatic mutations in mismatch repair genes, such as MSH 1, MSH 2 or MSH 6 [7].

During progression to a fully malignant, metastasising phenotype, additional mutations are required. Over time and during (chemo)therapy, further selective pressure will reflect in patterns of allelic loss or gain within the tumour, as well as in altered patterns of gene expression. It is estimated that finally, in a single cancer cell, a few hundred human genes have an altered expression. In a full-blown cancer cell, it is difficult to detect which mutations constitute early events.

3. To study the process of breast carcinogenesis

In order to unravel the multi-step process of breast carcinogenesis, a wide variety of (fresh, frozen or fixed) cancer material (cells, tissues and tissue sections; DNA and RNA samples) has been studied with a multitude of old and modern methods.

Cytogenetic abnormalities were first studied by karotype analyses and flow cytometry. Fluorescence in situ hybridisation (FISH) can also be used in fixed material, including paraffin sections and allows the detection of deletions and gains present in metaphase or interphase chromosomes. DNA ploidy status can be assessed reliably, but the limited availability of locus specific probes limits the even more interesting applications in studies of distinct chromosomal loci [8].

Loss of heterozygosity (LOH) analysis or micro-satellite analysis is based on the detection of allelic imbalances in small repetitive DNA sequences. When comparing normal to tumour material, LOH points to loss of one allele, and therefore also to the possible inactivation of genes located closely to the micro-satellite analysed [9].

Comparative genomic hybridisation (CGH) is a relative new technique that screens for losses and gains in human chromosomal material within the tumour DNA, using both fresh, frozen or fixed tissues, as well as isolated DNA. Although interpretation is not always simple, conclusions may be drawn from one single test about a possible activation of multiple oncogenes or inactivation of various tumour suppressor genes [10].

Gene expression, whether decreased or increased, at the RNA and protein level can be studied with various (semi) quantitative methods. Molecular biological techniques to detect mutations (sequencing) or altered expression of genes (micro arrays) are also of importance.

4. Genes involved in breast carcinogenesis

A long list of genes has been implied in breast cancer tumorigenesis. In the following, a short survey of the most important genes will be given (see Table 1).

4.1. Oncogenes

Many oncogenes, with different functionality and cellular localization, have been reported to play a role in human breast carcinogenesis. In sporadic breast cancer oncogene amplification is frequently found, but only a few of these amplified genes are crucial in the development of breast cancer, e.g. MYC, Int2, EMS1, CCND1 and ERBB2 [11–18]. Growth

Table 1

Gene	Locus	Role in hereditary breast cancer	Role in sporadic breast cancer	Reference(s)
BRCA1	17q12-21	Germline mutation (hereditary breast ovarian cancer syndrome)	Inactivation by hypermethylation of the BRCA1 promotor region	[2,23–30]
BRCA2	13q12-13	Germline mutation (hereditary breast ovarian cancer syndrome)	Silenced by overexpressed EMSY	[28,29,75]
TP53 (p53)	17p13.1	Germline mutation (Li-Fraumeni syndrome) TP53 mutations frequent in BRCA1 and BRCA2 mutant breast cancers	Late event	[31–36]
Rb1	13q14.1	No specific role	Late event	[30,37,38]
PTEN (MMAC1)	10q.23-24	Germline mutation (Cowden disease syndrome)	Rare	[39]
MYC	8q24	No specific role	Overexpressed in 25–30%	[11,30]
ERBB2/Her2/neu	17q21	Frequently underexpressed in BRCA1 mutant breast cancers	Overexpressed in 25–30%	[16]
CDH1 (E-Cadherin)	16q22.1	No specific role	Early event in lobular breast cancer	[44]
CCND1 (Cyclin D1)	11q13	Frequently underexpressed in BRCA1 mutant breast cancers	Overexpressed in 30–40%	[14,15,63]
ERα	6q25.1	Frequently underexpressed in BRCA1 mutant breast cancers	Underexpressed in 25%	[47,48,50,51]
ERβ	14q22-24	Not known	Not known	[49–51]

factors like EGF, TGFβ and IGF-1 could be also involved in proliferation and growth of breast cancer [19–22].

4.2. Tumour suppressor genes

The BRCA1 gene, located on chromosome 17q12-21, was cloned in 1994 [23]. BRCA1 is involved in many transcriptional processes. It has been associated with more than 15 different proteins involved in transcription, either in transcriptional activation or transcriptional repression [24]. It also plays a role in apoptosis. As a tumour suppressor, BRCA1 is a factor in maintaining genomic stability. It interacts with various proteins, and the complexes formed are involved in DNA recognition and repair [25,26].

Germline mutations in BRCA1 confer susceptibility to breast and ovarian cancer. Mutations of BRCA1 are scattered throughout the gene and consist of insertions, deletions, frameshifts, base substitutions and inferred regulatory mutations [2]. In sporadic breast cancer the gene is rarely mutated, but frequently functionally impaired [26–30].

The BRCA2 gene is located on chromosome 13q12-13. The gene codes for proteins involved in DNA repair, cell cycle control and transcription [28], and may have a function in terminal differentiation of breast epithelial cells [29].

In sporadic breast cancer, mutational inactivation of BRCA2 is rare as inactivation requires both gene copies to be mutated or totally lost [26,28,30].

The tumour suppressor gene p53 (TP 53), on chromosome 17p13.1, is one of the most frequently mutated genes in sporadic human cancer [30]. Most mutations are point mutations leading to proteins defective for sequence-specific DNA binding and activation of p53-responsive genes [31–33]. In sporadic breast carcinomas the occurrence of TP53 mutations is a late event. Rarely, a TP53 mutation is associated with hereditary breast cancer [33], as seen with the Li-Fraumeni Syndrome [34–36].

The Rb protein product of the retinoblastoma gene Rb1, located on chromosome 13q14.1, regulates the expression of BRCA1 via transcriptional activation. Double inactivation of both alleles of this tumour suppressor, due to mutations of the Rb1 gene, is found in hereditary and sporadic retinoblastoma. Rb1 mutations in the large-sized Rb1 locus are common in many cancers, including sporadic breast cancer but are probably late events [30,37,38].

The PTEN tumour suppressor gene is located on chromosome 10q23. Germline mutations play a role in

breast cancer (within the Cowden disease syndrome). Somatic mutations in sporadic breast cancer are rare [39].

The cell cycle checkpoint kinase CHEK2 gene (on chromosome 22) is a key mediator in DNA damage-response [40,41]. The 1100delC variant of the CHEK2 gene was found to cause low-penetrance susceptibility to familial breast cancer [42,43].

The CDH1 gene (on 16q22.1) encodes for the adhesion molecule E-Cadherin. In sporadic lobular breast cancer, CDH1 is claimed to behave as a tumour suppressor gene [44].

4.3. Apoptosis genes

Apoptosis or programmed cell death is a characteristic of normal, non-neoplastic cells. In cancer cells apoptosis is either abnormal or completely inhibited. Pro- and anti-apoptosis signals are under control of several genes, e.g. Bax, Bcl2 [45,46]. Sex hormones are known to either up-regulate or to down-regulate apoptotic genes [47].

4.4. Steroid receptors

The oestrogen receptor (ER) α gene located on chromosome 6q25.1, is the most important growth factor receptor involved in hormone-dependent breast carcinogenesis. The ERβ gene is located on 14q22-24.

Estrogens can act as tumour initiator, by causing direct DNA damage [48]. By induction of incessant mitosis, estrogens can promote accumulation of DNA replication damage ultimately leading to a malignant phenotype [47]. The two receptor isoforms are encoded by two different mRNAs, but share the same structural and functional domain composition [49]. Oestrogen receptors regulate gene expression by both oestrogen-dependent and oestrogen-independent mechanisms leading to activation of gene transcription, e.g. of cell cycle control proteins. These processes may result in cell proliferation. Overexpression of ERα is frequently observed in early stages of breast cancer [50]. The significance of ERβ in breast cancer is less clear than that of ERα. The presence of ERβ mRNA has been demonstrated both in normal as well as in malignant mammary gland tissue [51].

Progesterone receptor isoforms PR-A and PR-B have different physiological functions. Of 94 progesterone-regulated genes, 65 are uniquely regulated by PR-B, 4 uniquely by PR-A, and only 25 by both [52]. The role of PR-A and PR-B in breast tumorigenesis is not clear.

4.5. Invasion and cell adhesion genes

Invasion, cell adhesion and ‘homing’ of tumour cells are essential steps in the metastatic spread of cancer cells. Several genes are involved in this process, e.g. N-CAM, integrins, E-Cadherin, uPA, cathepsinD, B, collagenase I-IV, CD44, NME1 and metalloproteases [1,53].

4.6. Angiogenesis genes

Growth and progression of tumours is accompanied by neovascularisation (angiogenesis) [54]. Tumour cells in the stroma contribute to an increase of vascular endothelial growth factor (VEGF) and other angiogenic factors, like basic fibroblast growth factor and platelet-derived growth factor [55–57].

5. Current understanding of breast carcinogenesis

At present the development of a multi-step genetic model for breast carcinogenesis lags behind compared to that of colorectal cancer [58], which has been described as a process of serial step-wise accumulation of distinct genetic mutations.

As to the breast cancer model, there are a few uncertainties. Firstly, are hyperplasia (of the usual type), atypical hyperplasia and carcinoma in situ frequent and obligatory precursors of ductal or lobular breast cancer, or will cancer sometimes or mostly develop directly out of normal epithelium, without any precursor stage? Secondly, breast carcinogenesis is probably different in ductal and lobular carcinoma. And how would it be in other breast cancer types, such as tubular and medullar carcinomas? And finally, what are the differences between hereditary and sporadic breast cancer tumorigenesis? Complete answers to these questions are not available; our knowledge is still very limited and often fragmentary in nature [26,30,59].

Starting from various types of carcinoma in situ, it has been suggested that in sporadic breast cancer not one, but multiple, parallel running, progression

pathways exist [60–62]. Thus, poorly differentiated ductal carcinoma develops from grade 3 DCIS, with both lesions showing genetic amplifications in chromosomal region 17q12, while well-differentiated ductal carcinomas develop from well-differentiated DCIS, both being characterized by 16q loss, the locus of the E-Cadherin gene [61,62]. As a general rule, a mutation acquired early during tumorigenesis will be present in all later stages during cancer progression. Therefore, with a chromosomal loss (16q) early in the pathway to well-differentiated breast cancer, the concept that poorly differentiated ductal cancer results from dedifferentiation of a well differentiated tumour seems less likely.

Overexpression of Cyclin D is an early event that distinguishes all grades of DCIS from benign hyperplasic lesions [63]. High grade DCIS is furthermore often characterized by an early TP53-mutation [64] and ERBB2 overexpression [65], while low grade DCIS shows overexpression of E-Cadherin [66].

Invasive lobular cancer results from lobular carcinoma in situ, with an early loss of 16q and a gain in 1q, highly similar to the changes seen in well-differentiated DCIS. In mucinous carcinoma, amplification of 8q24 seems to be an early event [62,63]. Thus, with sophisticated methods such as CGH studies, early events in sporadic breast cancer are detected as allelic imbalances in 16q (the locus of the E-Cadherin gene), in 17q12 (the locus of the ERBB2/HER2-neu) and in 11q13 (the locus of the Cyclin D gene) [62,63,67,68].

Remarkably, any distinct genetic alteration associated with the important step of invasion has not been identified thus far [67,68]. However, it can be concluded that the most common genetic changes seen in invasive breast cancer already take place at the carcinoma in situ level. Here, the concept of carcinoma in situ probably differs substantially from that seen in adenocarcinoma of the colon, as well as from that seen in squamous cell carcinoma of the uterine cervix.

It is tempting to postulate that in sporadic breast tumorigenesis at least three different pathway-specific mechanisms of tumour progression can be discerned. Early events on these roads differ: loss of 16q (the pathway to G1 ductal tumours and lobular cancers) and gain in 17q12 and 8q (the pathway to G3 ductal cancers). One or even two different pathways might lead to G2 ductal tumours [63].

Thus, early in the multi-step process carcinogenesis, different breast cancer tumour types could emerge, probably with different cell biological characteristics (mitotic rate, apoptopic rate, ER expression, metastasing capability), and thus with different clinical outcomes.

Recent large-scale gene expression profiling using DNA microarray analysis techniques, has resulted in classification into clinically relevant breast cancer subgroups [69–71]. In one study, based on patterns of expression of over 500 selected genes, a subdivision into five distinct subtypes was obtained [71]. These five subtypes represent different biological entities and might originate from different cell types.

One of the five subtypes was characterized by overexpression of ERBB2 (located on 17q12) and poor prognosis. A second tumour type, lacking expression of the oestrogen receptor and also with a poor clinical prognosis, has been termed “basal”, as it resembles the pattern found in basal epithelial cells of the normal mammary gland. This basal tumour type differs from two other subtypes, namely luminal A and luminal B subtypes, which resemble cells that line the duct and give rise to the majority of breast cancers [71].

Remarkably, hereditary tumours of women with a BRCA1 gene mutation are classified exclusively as basal subtype tumours, and those of BRCA2 mutation carriers as luminal A subtype tumours [69,71]. In general, the latter subtype has a better survival than the basal subtype. As both subtypes are also found in (non-BRCA1 or 2 mutation carriers with) sporadic breast cancer, the question can be asked as to the role of BRCA1 and 2 in sporadic carcinogenesis. In sporadic breast cancer, mutational inactivation of BRCA1 or BRCA2 genes is a rare event [30]. However, non-mutational disregulation or suppression of BRCA1 function [25–30,72–74] and of the BRCA2 function [75] has been described. Various mechanisms could play a role, such as hypermethylation of the BRCA1 promoter [74] or binding of BRCA2 by EMSY [75].

EMSY is a novel gene product, which binds to exon 3 of the BRCA2 gene, and thereby suppresses the transactivational activity of BRCA2. EMSY maps to the chromosome 11q13 locus (together with the Cyclin D1 gene) and encodes a protein of 1322 amino acids, with no obvious function. The EMSY gene is amplified in a subset of sporadic breast cancers, around

13% of the total, which thus could lead to inactivation of the BRCA2 pathway in sporadic tumours, mimicking the effect of BRCA2 inactivation after familial BRCA2 deletion in hereditary breast cancers.

Although it seems evident that EMSY represses transcriptional activation of BRCA2, it is not certain that the overexpression of EMSY would be sufficient to drive tumorigenesis in sporadic breast cancer. Also, there is uncertainty as to the role of EMSY in hereditary breast tumours that already have one defective BRCA2 allele [76].

Distinct somatic genetic changes have been found to be associated with tumour progession in carriers of BRCA1 and BRCA2 germline mutation carriers [59]. These somatic genetic mutations found in hereditary breast cancers seem to be both quantitatively and qualitatively different from those involved in sporadic breast cancer progression. In BRCA1 tumours that are generally ER-negative and strongly infiltrated by tumour lymphocytes [69,70], TP53 mutation [59,33] is very frequent. TP53 is mutated also frequently in BRCA2 mutation carriers. This is remarkable as p53 mutations, common in most solid tumours, are less common in sporadic breast cancers. Also, TP53 mutations identified in BRCA1 and BRCA2 mutation carriers are in general of a different nature than those reported in sporadic human cancers [33].

The pathway to cancer starts in hereditary breast cancer from a germline pathological mutation in one of the tumour suppressor genes. In normal breast tissue of BRCA1 or BRCA2 mutation carriers an altered expression of oestrogen response proteins is found, together with a predominance of progesterone receptor A [77]. This could lead to changes in progesterone signalling in this hormone-dependent tissue and thus to an increased risk for cancer. An early event in hereditary breast cancer is the inactivation of the second allele in addition to the germline mutation present in the first allele. Here, cancer develops through loss of function of both the maternal and paternal alleles of a tumour suppressor gene. The loss of the second allele may occur after some time as a result of exogenous toxins or just by chance during DNA replication.

This so-called “two-hit”-model of carcinogenesis, originally suggested by Knudson [78] applies to tumour suppressor genes and DNA repair genes. The loss of the second still functional allele (reflected in a loss of heterozygosity) starts cancer development far more often in hereditary breast cancers than in sporadic breast cancer. In this sense, the pathway to invasive breast cancer starts in women, who have already a germline mutation in one of the cancer susceptibility genes, from an early event that is highly different from that found in sporadic cancer.

It can be assumed that the specific early events during tumorigenesis would also have implications for subsequent, specific, somatic, genetic defects that must accumulate during further progression to a malignant phenotype, as indeed have been found in some studies [59,79].

Based on our current understanding, which is far from complete, the following can nevertheless be concluded. Oncogenic pathways leading from early events to invasive, metastasising cancers are different for sporadic versus hereditary breast cancer. In hereditary cancers tumorigenesis most often starts from the inactivation of two alleles of a tumour suppressor gene, while in sporadic cancer more often genomic amplification of only one allele of an oncogene would be an early event.

Both in hereditary cancer as well as in sporadic cancer, early events can take place in a variety of specific genes, leading through different pathways, probably to different tumour types, which have a different clinical outcome.

Knowledge of the different tumour development pathways, each starting from distinct and specific early events leading to specific clinically relevant subtype of tumours, would also be of value for the clinic. Gene expression profiling could help to identify patients with different breast cancer subtypes, and therefore, different therapeutic options.

References

[1] Beckmann WM, Niederacher D, Schnürch H-G, Gusterson BA, Bender HG. Multistep carcinogenesis of breast cancer and tumour heterogeneity. J Mol Med 1997;75:429–39.

[2] Wooster R, Weber BL. Breast and ovarian cancer. N Engl J Med 2003;348:2339–47.

[3] Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:843–54.

[4] Clemons M, Goss P. Estrogen and the risk of breast cancer. N Engl J Med 2001;344:276–85.
[5] Osin PP, Anbazhagan R, Bartkova J, Nathan B, Gusterson BA. Breast development gives insights into breast disease. Histopathology 1998;33:275–83.
[6] O'Connell P. Genetic and cytogenetic analyses of breast cancer yield different perspectives of a complex disease. Breast Cancer Res Treat 2003;78:347–57.
[7] Balogh GA, Russo IH, Russo J. Mutations in mismatch repair genes are involved in the neoplastic transformation of human breast epithelial cells. Int J Oncol 2003;23:411–9.
[8] Patterson AH, McManus DT, Maxwell P. Detection of chromosomal numerical abnormalities in clinical breast tumour fine-needle aspirations by fluorescence in situ hybridisation (FISH): refinement of a method. Br J Biomed Sci 1998;55:2–7.
[9] Tong D, Schuster E, Czerwenka K, Leodolter S, Zeillinger R. Loss of heterozygosity on chromosome 13q: suggestion of a candidate tumor suppressor gene in sporadic breast cancer. Breast Cancer Res Treat 2004;83:143–8.
[10] Bürger H, Schmidt H, Beckmann A, Zänker KS, Böckner W, Brandt H. Genetic characterisation of invasive breast cancer—a comparison of CGH and PCR-based multiplex microsatellite analysis. J Clin Pathol 2001;54:836–40.
[11] Nass SJ, Dickson RB. Defining a role for c-Myc in breast tumorigenesis. Breast Cancer Res Treat 1997;44:1–22.
[12] Ormandy CJ, Musgrove EA, Hui R, Daly RJ, Sutherland RL. Cyclin D1, EMS1 and 11q13 amplification in breast cancer. Breast Cancer Res Treat 2003;78:323–35.
[13] Hosokawa Y, Arnold A. Mechanism of cyclin D1 (CCND1, PRAD1) overexpression in human cancer cells: analysis of allele-specific expression. Genes Chromosomes Cancer 1998;22:66–71.
[14] Barnes DM, Gillett CE. Cyclin D1 in breast cancer. Breast Cancer Res Treat 1998;52:1–15.
[15] Steeg PS, Zhou Q. Cyclins and breast cancer. Breast Cancer Res Treat 1998;52:17–28.
[16] Miles DW, Harris WH, Gillett CE, Smith P, Barnes DM. Effect of c-erbB(2) and estrogen receptor status on survival of women with primary breast cancer treated with adjuvant cyclophosphamide/methotrexate/fluorouracil. Int J Cancer 1999;84:354–9.
[17] Fioravanti L, Cappelletti V, Coradini D, et al. Int-2 oncogene amplification and prognosis in node-negative breast carcinoma. Int J Cancer 1997;74:620–4.
[18] An HX, Niederacher D, Dominik SI, et al. Int-2 and c-erbB-2 gene amplification detected in 70 frozen human breast carcinomas by quantitative polymerase chain reaction. Anticancer Res 1997;17:3133–6.
[19] Earp 3rd HS, Calvo BF, Sartor CI. The EGF receptor family—multiple roles in proliferation, differentiation, and neoplasia with an emphasis on HER4. Trans Am Clin Climatol Assoc 2003;114:315–33.
[20] Druckmann R, Rohr UD. IGF-1 in gynaecology and obstetrics: update 2002. Maturitas 2002;41(S1):S65–83.
[21] Sachdev D, Yee D. The IGF system and breast cancer. Endocr Relat Cancer 2001;8:197–209.
[22] Barcellos-Hoff MH. Latency and activation in the control of TGF-beta. J Mammary Gland Biol Neoplasia 1996;1:353–63.
[23] Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994;266:66–71.
[24] Cable PL, Wilson CA, Calzone FJ, et al. Novel consensus DNA-binding sequence for BRCA1 protein complexes. Mol Carcinog 2003;38:85–96.
[25] Jhanwar-Uniyal M. BRCA1 in cancer, cell cycle and genomic stability. Front Biosci 2003;8:S1107–17.
[26] Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 2002;108:171–82.
[27] Lambie H, Miremadi A, Pinder SE, et al. Prognostic significance of BRCA1 expression in sporadic breast carcinomas. J Pathol 2003;200:207–13.
[28] Kerr P, Ashworth A. New complexities for BRCA1 and BRCA2. Curr Biol 2001;11:R668–76.
[29] Vidarsson H, Mikaelsdottir EK, Rafnar T, et al. BRCA1 and BRCA2 bind Stat5a and suppress its transcriptional activity. FEBS Lett 2002;532:247–52.
[30] Lerebours F, Lidereau R. Molecular alterations in sporadic breast cancer. Crit Rev Oncol Hematol 2002;44:121–41.
[31] Ko LJ, Prives C. p53: puzzle and paradigm. Genes Dev 1996;10:1054–72.
[32] Sigal A, Rotter V. Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. Cancer Res 2000;60:6788–93.
[33] Gasco M, Yulug IG, Crook T. TP53 mutations in familial breast cancer: functional aspects. Hum Mutat 2003;21:301–6.
[34] Santibanez-Koref MF, Birch JM, Hartley AL, et al. p53 germline mutations in Li-Fraumeni syndrome. Lancet 1991; 338:1490–1.
[35] Law JC, Strong LC, Chidambaram A, Ferrell RE. A germ line mutation in exon 5 of the p53 gene in an extended cancer family. Cancer Res 1991;51:6385–7.
[36] Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. Hum Mutat 2003;21:313–20.
[37] Sherr CJ. Cancer cell cycles. Science 1996;274:672–7.
[38] Wang A, Schneider-Broussard R, Kumar AP, MacLeod MC, Johnson DG. Regulation of BRCA1 expression by the Rb-E2F pathway. J Biol Chem 2000;275:4532–6.
[39] Ueda K, Nishijima M, Inui H, et al. Infrequent mutations in the PTEN/MMAC1 gene among primary breast cancers. Jpn J Cancer Res 1998;89:17–21.
[40] Rouse J, Jackson SP. Interfaces between the detection, signalling, and repair of DNA damage. Science 2002;297:547–51.
[41] Myung K, Datta A, Kolodner RD. Suppression of spontaneous chromosomal rearrangements by S phase checkpoint functions in *Saccharomyces cerevisiae*. Cell 2001;104:397–408.
[42] Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. Low-penetrance susceptibility to breast cancer due to CHEK2(,)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 2002;31:55–9.
[43] Vahteristo P, Bartkova J, Eerola H, et al. A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. Am J Hum Genet 2002;71:432–8.

[44] Berx G, Cleton-Jansen AM, Nollet F, et al. E-Cadherin is a tumour/invasion suppressor gene mutated in human lobular breast cancers. EMBO J 1995;14:6107–15.
[45] Makin G, Dive C. Recent advances in understanding apoptosis: new therapeutic opportunities in cancer chemotherapy. Trends Mol Med 2003;9:251–5.
[46] Liu W, Bulgaru A, Haigentz M, Stein CA, Perez-Soler R, Mani S. The BCL2-family of protein ligands as cancer drugs: the next generation of therapeutics. Curr Med Chem Anticancer Agents 2003;3:217–23.
[47] Kenemans P, Bosman A. Breast cancer and post-menopausal hormone therapy. Best Pract Res Clin Endocrinol Metab 2003;17:123–37.
[48] Liehr JG. Dual role of oestrogens as hormones and pro-carcinogens: tumour initiation by metabolic activation of oestrogens. Eur J Cancer Prev 1997;6:3–10.
[49] Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 1996;93:5925–30.
[50] Hayashi SI, Eguchi H, Tanimoto K, et al. The expression and function of estrogen receptor alpha and beta in human breast cancer and its clinical application. Endocr Relat Cancer 2003;10:193–202.
[51] Cullen R, Maguire TM, McDermott EW, Hill AD, O'Higgins NJ, Duffy MJ. Studies on oestrogen receptor-alpha and -beta mRNA in breast cancer. Eur J Cancer 2001;37:1118–22.
[52] Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem 2002;277:5209–18.
[53] Sledge Jr GW, Miller KD. Exploiting the hallmarks of cancer: the future conquest of breast cancer. Eur J Cancer 2003;39:1668–75.
[54] Boudreau N, Myers C. Breast cancer-induced angiogenesis: multiple mechanisms and the role of the microenvironment. Breast Cancer Res 2003;5:140–6.
[55] Yoshiji H, Gomez DE, Shibuya M, Thorgeirsson UP. Expression of vascular endothelial growth factor, its receptor, and other angiogenic factors in human breast cancer. Cancer Res 1996;56:2013–6.
[56] Relf M, LeJeune S, Scott PA, et al. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 1997;57:963–9.
[57] Fukumura D, Xavier R, Sugiura T, et al. Tumor induction of VEGF promoter activity in stromal cells. Cell 1998;94:715–25.
[58] Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
[59] Tirkkonen M, Johannsson O, Agnarsson BA, et al. Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. Cancer Res 1997;57:1222–7.
[60] Bürger H, Otterbach F, Simon R, et al. Comparative genomic hybridization of ductal carcinoma in situ of the breast-evidence of multiple genetic pathways. J Pathol 1999;187:396–402.
[61] Bürger H, Simon R, Schäfer K-L, et al. Genetic relationship of lobular carcinoma in situ, ductal carcinoma in situ and invasive carcinoma of the breast. J Clin Pathol: Mol Pathol 2000;53:118–21.
[62] Bürger H, Mommers EC, Littman R, et al. Ductal invasive G2 and G3 carcinomas of the breast are the end stage of at least two different lines of genetic evolution. J Pathol 2001;194:165–70.
[63] Weinstat SD, Merino MJ, Manrow RE, et al. Overexpression of cyclin D mRNA distinguishes invasive and in situ breast carcinomas from non-malignant lesions. Nat Med 1995;1:1257–60.
[64] Done SJ, Arneson NC, Ozcelik H, Redston M, Andrulis IL. p53 mutations in mammary ductal carcinoma in situ but not in epithelial hyperplasias. Cancer Res 1998;58:785–9.
[65] Quinn CM, Ostrowski JL, Harkins L, Rice AJ, Loney DP. Loss of bcl-2 expression in ductal carcinoma in situ of the breast relates to poor histological differentiation and to expression of p53 and c-erbB-2 proteins. Histopathology 1998;33:531–6.
[66] Gupta SK, Douglas JA, Jasani B, Morgan JM, Pignatelli M, Mansel RE. E-Cadherin (E-cad) expression in duct carcinoma in situ (DCIS) of the breast. Virchows Arch 1997;430: 23–8.
[67] Richard F, Pacyna-Gengelbach M, Schluns K, et al. Patterns of chromosomal imbalances in invasive breast cancer. Int J Cancer 2000;89:305–10.
[68] Kuukasjarvi T, Tanner M, Pennanen S, Karhu R, Kallioniemi OP, Isola J. Genetic changes in intraductal breast cancer detected by comparative genomic hybridization. Am J Pathol 1997;150:1465–71.
[69] van't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–6.
[70] van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999–2009.
[71] Sørlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 2003;100:8418–23.
[72] Fraser JA, Reeves JR, Stanton PD, et al. A role for BRCA1 in sporadic breast cancer. Br J Cancer 2003;88:1263–70.
[73] Rosen EM, Fan S, Pestell RG, Goldberg ID. BRCA1 gene in breast cancer. J Cell Physiol 2003;196:19–41.
[74] Dobrovic A, Simpfendorfer D. Methylation of the BRCA1 gene in sporadic breast cancer. Cancer Res 1997;57:3347–50.
[75] Hughes-Davies L, Huntsman D, Ruas M, et al. EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. Cell 2003;115:523–35.
[76] King MC. A novel BRCA2-binding protein and breast and ovarian tumorigenesis. N Engl J Med 2004;350:1252–3.
[77] Mote PA, Leary JA, Avery KA, et al. kConFab Investigators. Germ-line mutations in BRCA1 or BRCA2 in the normal

breast are associated with altered expression of estrogen-responsive proteins and the predominance of progesterone receptor A. Genes Chromosomes Cancer 2004;39:236–48.
[78] Knudson Jr AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 1971;68:820–3.
[79] Wessels LF, van Welsem T, Hart AA, van't Veer LJ, Reinders MJ, Nederlof PM. Molecular classification of breast carcinomas by comparative genomic hybridization: a specific somatic genetic profile for BRCA1 tumors. Cancer Res 2002;62:7110–7.