DNA DAMAGE RESPONSE GENES AND CHROMOSOME 11q21-q24 CANDIDATE TUMOR SUPPRESSOR GENES IN BREAST CANCER

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OULU 2002

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Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium 9 of the University Hospital of Oulu, on May 31st, 2002, at 12 noon.

OULUN YLIOPISTO, OULU 2002

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Reviewed by
Docent Anne Kallioniemi
Docent Päivi Peltomäki

ISBN 951-42-6714-1 (URL: http://herkules.oulu.fi/isbn9514267141/)

ALSO AVAILABLE IN PRINTED FORMAT
Acta Univ. Oul. D 678, 2002
ISBN 951-42-6713-3
ISSN 0355-3221 (URL: http://herkules.oulu.fi/issn03553221/)

OULU UNIVERSITY PRESS
OULU 2002

Allinen, Minna, DNA damage response genes and chromosome 11q21-q24 candidate tumor suppressor genes in breast cancer

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Oulu, Finland
2002

Abstract

As the defects in DNA repair and cell cycle control are known to promote tumorigenesis, a proportion of inherited breast cancers might be attributable to mutations in the genes involved in these functions. In the present study, three such genes, *TP53*, *CHK2* and *ATM*, which are also associated with known cancer syndromes, were screened for germline mutations in Finnish breast cancer patients.

In combination with our previous results, three *TP53* germline mutations, Tyr220Cys, Asn235Ser and Arg248Gln, were detected in 2.6% (3/108) of the breast cancer families. The only observed *CHK2* alteration with a putative effect on cancer susceptibility, Ile157Thr, segregated ambiguously with the disease, and was also present in cancer-free controls. The available functional data, however, suggests that the altered CHK2 in some way promote tumorigenesis. Furthermore, compared to the other studied populations, Ile157Thr seems to be markedly enriched in Finland. Thus, the clinical significance of Ile157Thr requires further investigation among Finnish cancer patients.

ATM germline mutations appear to contribute to a small proportion of the hereditary breast cancer risk, as two distinct *ATM* mutations, Ala2524Pro and 6903insA, were found among three families (1.9%, 3/162) displaying breast cancer. They all originated from the same geographical region as the AT families with the corresponding mutations, possibly referring to a founder effect concerning the distribution of these mutations in the Finnish population.

The genes important for tumorigenesis in sporadic disease might also contribute to familial breast cancer. Therefore, four putative LOH targets genes in chromosome 11q21-q24 were screened for intragenic mutations, and five were analyzed for epigenetic inactivation in sporadic breast tumors. The lack of somatic intragenic mutations in *MRE11A*, *PPP2R1B*, *CHK1* and *TSLC1* led us next to investigate promoter region hypermethylation as a mechanism capable of silencing these genes, as well as the *ATM* gene. Only *TSLC1* demonstrated involvement of CpG island methylation, which was especially prominent in three tumors. This suggests that together with LOH, methylation could result in biallelic inactivation of the *TSLC1* gene in breast cancer.

Keywords: breast cancer susceptibility, double-strand break signaling, CpG island methylation

To Paavo

Acknowledgements

The present study has been carried out at the Department of Clinical Genetics, Oulu University Hospital and University of Oulu, during the years 1999-2002.

I owe my sincere thanks to Professor Jaakko Leisti, the head of the Department of Clinical Genetics, for the opportunity to work in the field of cancer research.

I am deeply grateful to my supervisor, Docent Robert Winqvist. Through his unfailing support and innovative guidance during these years, he has provided me with unforgettable sights to the world of cancer genetics.

I wish to express my gratitude to Docent Anne Kallioniemi and Docent Päivi Peltomäki, for the invaluable comments on the manuscript of this thesis.

I warmly thank Sirkka-Liisa Leinonen for the careful revision of the language of this manuscript, and Pirkko Ranta-aho for the numerous helpful discussions on scientific writing in English. I also wish to thank Tuomo Toukomies for his assistance with scientific illustrations.

I wish to acknowledge all the collaborators and co-authors, Professor Guillermo Blanco, Professor Anne-Lise Børresen-Dale, Professor Hannaleena Eerola, Laila Jansen, M.Sc., Tommi Kainu, Ph.D., Professor Olli-Pekka Kallioniemi, Docent Helena Kääriäinen, Kirsten Laake, Ph.D., Samu Mäntyniemi, M.Sc., Docent Heli Nevanlinna, Katrin Rapakko, B.Sc., Kirsi Syrjäkoski, M.Sc., Pia Vahteristo M.Sc., and Docent Kirsi Vähäkangas.

I am most grateful to Pia Huusko and Virpi Launonen, for helping me on my feet at the very beginning of this project, and for always stressing the importance of the right attitude towards research. I also wish to acknowledge your priceless comments and suggestions concerning the original papers and this thesis.

My warmest thanks go to Kati Outila and Liisa Peri, for it was great working with you. During the most intense period of the project, we did not only redefine the word ‘teamwork’ by adapting an efficient, almost symbiotic working pattern, but always had fun, being loud and never got enough of ABBA. And Kati, besides being one in a million as a colleague, I owe a special thank you for a fantastic friend. All the refreshing recreational activities outside the laboratory, especially during the final months of the project, truly gave an interesting twirl to the exhausting daily routine.

I am also grateful to all my former and present colleagues at the laboratory, Katri ‘T’ Heikkinen, Sanna-Maria Karppinen, Marika Kujala, Sonja Kujala, Jaana Lahti-Domenici, Mari Laiti, Katrin Rapakko and Christina Saarimaa. Furthermore, the staff at the Department of Clinical Genetics deserves my warmest thanks for their assistance and optimism over these years.

I wish to express special thanks to all my dearest friends: Satu and Tommi, Virve and Hanna, for all the pleasant moments in the world outside the laboratory, Julie for her optimism and belief that I would eventually be cured from the ‘Rebecca-disease’, and Ziggy for the amazing times we shared in Rockville.

Finally, I owe my loving thanks to my family, parents Ritva and Jaakko, and the Allinen Brothers; Timo, Juha, Jouni and Tuomo. No matter how crazy decisions I made, you never questioned my choices, but greeted them with the sincerest support.

This study has been supported financially by the University of Oulu, Oulu University Hospital, the Cancer Society of Northern Finland, the Finnish Cancer Foundation, the Finnish Breast Cancer Group, Academy of Finland and Farmos Research and Science Foundation, which I gratefully acknowledge.

May 2002, Oulu Minna Allinen

Abbreviations

AT	ataxia-telangiectasia
ATM	ataxia-telangiectasia mutated
ATR	ataxia-telangiectasia and Rad3-related
BASC	BRCA1-associated genome surveillance complex
BCL2	B-cell chronic lymphatic leukemia/lymphoma 2 oncogene
BER	base excision repair
BRCA1	breast cancer 1 gene
BRCA2	breast cancer 2 gene
CC	cell cycle
CDC	cell division cycle -protein
CDK	cyclin-dependent kinase
cDNA	complementary DNA
CGH	comparative genomic hybridisation
CHK1	cell cycle checkpoint kinase 1 gene
CHK2	cell cycle checkpoint kinase 2 gene
CpG	cytosine-guanine dinucleotide
CSGE	conformation-sensitive gel electrophoresis
DNA	deoxyribonucleic acid
DNMT	DNA methyl transferase
DSB	double-strand break
FHA	forkhead associated
G1	gap between mitosis and the onset of DNA replication
G2	gap between DNA synthesis and the onset of mitosis
GGR	global genome repair
GSTP1	glutathione S-transferase P1 gene
HNPCC	hereditary nonpolyposis colorectal cancer
HR	homologous recombination
kb	kilobase
kDa	kilodalton
LFL	Li-Fraumeni-like
LFS	Li-Fraumeni syndrome

LOH	loss of heterozygosity
MMR	mismatch repair
MLH1	mutL (*E.coli*) homologue 1 gene
MLH3	mutL (*E.coli*) homologue 3 gene
MRE11	meiotic recombination protein 11 (*S.cerevisiae*) homologue
MRE11A	gene for MRE11 protein
MSH2	mutS (E.coli) homologue 2 gene
MSH6	mutS (E.coli) homologue 6 gene
NBS	Nijmegen breakage syndrome
NBS1 (*nibrin, p95*)	Nijmegen breakage syndrome gene
NER	nucleotide excision repair
NHEJ	non-homologous end-joining
p	short arm of the chromosome
p21 (WAF1, CDKN1A)	cyclin-dependent kinase inhibitor 1A
p53	tumor protein 53
PCR	polymerase chain reaction
PMS2	postmeiotic segregation increased (*S.cerevisiae*)-like 2 gene
PP2A	serine-theronine protein phosphatase 2A
PPP2R1B	gene encoding the β isoform of the regulatory subunit of serine/threonine protein phosphatase 2A
q	long arm of the chromosome
RAD50	*S.cerevisiae* RAD50 homolog
RNA	ribonucleic acid
S	DNA synthesis of the cell cycle
TCR	transcription coupled repair
TP53	gene for tumor protein 53
TSG	tumor suppressor gene
TSLC1 (IGSF4)	tumor suppressor in lung cancer 1
UV	ultra violet

List of original articles

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Rapakko K*, Allinen M*, Syrjäkoski K, Vahteristo P, Huusko P, Vähäkangas K, Eerola H, Kainu T, Kallioniemi O-P, Nevanlinna H & Winqvist R (2001) Germline *TP53* alterations in Finnish breast cancer families are rare and occur at conserved mutation-prone sites. Br J Cancer 84: 116-119.

II Allinen M, Huusko P, Mäntyniemi S, Launonen V & Winqvist R (2001) Mutation analysis of the *CHK2* gene in families with hereditary breast cancer. Br J Cancer 85: 209-212.

III Allinen M, Launonen V, Laake K, Jansen L, Huusko P, Kääriäinen H, Børresen-Dale A-L & Winqvist R (2002) *ATM* mutations in Finnish breast cancer patients. J Med Genet 39: 192-196.

IV Allinen M, Peri L, Kujala S, Lahti-Domenici J, Outila K, Karppinen S-M, Launonen V & Winqvist R (2002) Analysis of 11q21-24 loss of heterozygosity candidate target genes in breast cancer: indications of *TSLC1* promoter hypermethylation. Genes Chrom Cancer. In press.

*The authors contributed equally to the study

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1 Introduction

Apart from being the most common malignancy in women worldwide (Parkin *et al.* 1993), breast cancer is also one of the most extensively investigated human cancers. The genetic background of cancer predisposition, tumor initiation, and the malignant transformation ultimately leading to a metastatic disease are all widely addressed topics, and understanding of the genetic defects underlying these fundamental processes is expected to unravel new possibilities for cancer treatment and prevention.

Mutations linked to an inherited cancer predisposition are usually found in tumor suppressor genes (TSG), which often encode proteins involved in cell cycle regulation and the maintenance of genomic integrity. In breast cancer research, a significant leap forward was taken with the identification of the two major breast cancer susceptibility genes, *BRCA1* and *BRCA2* (Miki *et al.* 1994, Wooster *et al.* 1995), which both have important functions in DNA double-strand break (DSB) repair (Liu & West 2002 and refs. therein). By genetic linkage and mutation analysis, it was shown that the lifetime risk of breast cancer is notably elevated in people carrying germline mutations in these genes (Ford *et al.* 1998). Further, mutations in TSGs linked to inherited syndromes with increased cancer predisposition, e.g. *TP53*, *ATM, PTEN, LKB1* and *AR* (Swift *et al.* 1987, Malkin *et al.* 1990, Srivastava *et al.* 1990, Wooster *et al.* 1992, Lobaccaro *et al.* 1993, Tsou *et al.* 1997, Boardman *et al.* 1998), seem to explain a small additional fraction of inherited breast cancer susceptibility. Despite these findings, however, the genes behind the increased disease susceptibility have remained unidentified in a large proportion of inherited breast cancers.

Subtle sequence variants in low-penetrance genes have been hypothesized to confer a clearly higher attributable risk of breast cancer in the general population than the much more rare mutations in high-penetrance cancer susceptibility genes (Nathanson & Weber 2001). Because genetic linkage studies are expected to identify only moderate or high-penetrance susceptibility genes, other approaches are currently utilized to find and evaluate the contribution of genetic variants in putative low-penetrance genes. These candidates are often chosen for analysis based on their essential biological functions. For instance, case-control studies of several genes involved in a variety of cellular pathways essential to DNA repair and cell cycle control are being performed. Furthermore, genetic alterations which have an effect on cancer risk in *BRCA1* and *BRCA2* mutation carriers

might alone or in combination with alterations in other genes, such as *RAD51* and *HRAS*, for example, slightly increase the risk even in non-carriers (Phelan *et al.* 1996, Rebbeck *et al.* 1999, Nathanson & Weber 2001, Levy-Lahad *et al.* 2001).

Alternatively, genes harboring the defects leading to cancerous cell growth can be uncovered by studying somatic mutations. According to Knudson's 'two-hit' hypothesis, the inactivation of a TSG requires two independent events (Knudson 1971). Loss of heterozygosity (LOH) within a certain narrow chromosomal region is usually recognized as a hallmark of a putative TSG (Cavenee *et al.* 1983). If one functional allele is inactivated as a result of chromosomal deletion, for example, the other copy can be subsequently silenced by either a genetic or an epigenetic alteration. However, if one of the alleles harbors an inherited mutation, even a single event can be sufficient to unmask the two inactivating alteration and thus result in biallelic silencing of the tumor suppressor gene (Kinzler & Vogelstein 1997, Jones & Laird 1999).

In this study, the focus was on assessing the contribution of germline mutations in selected DSB signaling and DNA repair genes associated with inherited cancer syndromes to breast cancer susceptibility in Finland. Mutation analysis of three such genes, *TP53*, *CHK2* and *ATM*, was performed both in cancer families and in an appropriate control population. Additionally, in a cohort of sporadic breast cancer patients exhibiting LOH on chromosome 11q21-q24, candidate tumor suppressor genes were analyzed for intragenic alterations as well as promoter region hypermethylation, an epigenetic mechanism of gene silencing.

2 Review of the literature

2.1 Genetic aberrations in cancer

Cancer is a multistage process, and three to seven successive mutations are estimated to be required for the malignant conversion of a normal cell (Miller 1980, Weinberg 1989, Vogelstein & Kinzler 1993, Kinzler & Vogelstein 1996). Thus, as the mutation rate is typically of the order of less than 10^{-6} per each cell division, the likelihood of a single cell to accumulate the requisite number of independent mutations to inactivate the cellular defenses against the development of cancer appears to be very low (Loeb 1991). However, there are two general mechanisms that make the series of cancer-promoting mutations more likely: mutations enhancing the cell proliferation rate increase the size of the target population of cells where the next mutation may occur, and mutations affecting the stability of the genome increase the overall mutation rate (Strachan & Reed 1999).

The multistep nature of cancer has been most extensively studied in colorectal cancer (Fearon & Vogelstein 1990, Kinzler & Vogelstein 1996). Pathogenic mutations usually occur in the genes controlling the cell cycle, apoptosis and genomic integrity. Genetic lesions may also influence proteins, which are responsible for cell-to-cell contacts, or the factors needed for tumor expansion and invasion into nearby tissues. Proto-oncogenes and tumor suppressor genes (TSG) compose the two main categories of aberrantly functioning genes in cancer.

The normal activity of a proto-oncogene supports cell proliferation, but a gain of function mutation (e.g. amplification, point mutation, or transposition to an active chromosome domain) in a single allele may result in inappropriate or excessive activity (Park 1998 and refs. therein). Tumor suppressor gene products, on the other hand, are targeted to inhibit events typical of cancerous behavior. They are responsible for controlling the cell cycle progression and the induction of apoptosis and for maintaining genomic integrity by ensuring accurate replication, repair and segregation of the cell's DNA. Inactivation of both alleles of a TSG may leave some of these crucial regulatory functions uncontrolled, and thus provide the mutated cell with a growth advantage (Fearon 1998 and refs. therein). The germline alterations related to inherited cancer susceptibility are frequently found in tumor suppressor genes, and only on a few

occasions (*RET*, *CDK4* and *MET*) have they been found in proto-oncogenes (Mulligan *et al.* 1993, Zuo *et al.* 1996, Schmidt *et al.* 1997). Conversely, cancer-associated somatic mutations are found in genes belonging to both categories.

Apart from the sequential tumor progression pathway, an alternative model has also been described (Shibata & Aaltonen 2001). In the new branching model, the final clonal expansion arises from a single founder cell, which is the only survivor among the other dead-end lineages. Therefore, the mutations in the developing tumor fall into two groups: mutations acquired by the final founder cell are present in every cell, whereas mutations arising during clonal expansion are found in only a fraction of malignant cells.

2.2 Clinicopathological profile of breast cancer

About 95% of all breast cancers arise from the epithelium of the mammary gland, including the terminal ducts and lobules. The transition of normal breast epithelium to a malignant tumor occurs in several stages. First, normal cells become hyperplastic, and some of these cells with atypical appearance subsequently go through malignant transformation and give rise to a carcinoma. A noninvasive carcinoma (carcinoma *in situ*) may eventually develop into an invasive carcinoma with metastatic potential. Ductal (70%) and lobular (about 6%) cancers are the two largest histological subgroups of invasive breast cancer. Rare histological subtypes include medullar, mucinous, papillary and tubular carcinomas, as well as Paget's disease (Berg & Hutter 1995).

At present, the most powerful factors for assessing the prognosis of breast cancer are tumor size and differentiation (histological type and grade), lymph node stage and vascular invasion (reviewed in Elston *et al.* 1999). The clinical TNM staging of a tumor is based on the above-mentioned factors, and the stage of a tumor is defined at the time of diagnosis. The 10-year relative survival in patients with stage 0 tumors (carcinoma *in situ*) is 95% and decreases in each class as follows: stage I (diameter 2 cm or less, axillary nodes not involved), 88%; stage II (diameter between 2 to 5 cm and/or mobile axillary nodes), 66%; stage III (larger than 5 cm and/or fixed axillary nodes), 36%; stage IV (distant metastases), 7% (Bland *et al.* 1998).

The response to endocrine therapy can be predicted by evaluating the estrogen receptor (ER) and progesterone receptor (PgR) status. While only one-third of unselected breast cancer patients will respond to anti-estrogen therapy, the response rate in ER-positive tumors is approximately 50%. Combined with positive PgR status, almost 80% of patients will respond to endocrine therapy (NIH Consensus Development Conference 1980).

Besides the traditional indicators for assessing prognosis, many molecular markers have been proposed as putative prognostic factors. Among the most promising ones are the urokinase plasminogen activator (uPA) and its inhibitor PAI-1, which participate in apoptosis through matrix degradation (Liotta *et al.* 1981, Foekens *et al.* 1994). The prognostic information of uPA seems to be independent of the traditional morphological factors (Duffy *et al.* 1999). Amplification and overexpression of the ERBB2 proto-oncogene occurs in 10-34% of breast cancers, and generally correlates with a poor

outcome (Ross & Fletcher 1999). These patients with tumors overexpressing ERBB2 can be treated with a therapeutic antibody, herceptin, and as a single agent, the treatment produces a response rate of approximately 15% (Cobleigh *et al.* 1999). Furthermore, administration of herceptin with chemotherapy results in better response rates that chemotherapy alone (Shaks 1999). In addition, *TP53* gene mutations, expression of E-cadherin, epidermal growth factor (EGF), Ki67 and BCL2 as well as the microvessel count have all been shown to possess prognostic importance (Dowsett 1998, Duffy 2001).

Although the markers mentioned above are significant in univariate analysis, many of them lose their prognostic values in multivariate analysis because of internal correlations (Howat *et al.* 1983, Battaglia *et al.* 1988). In a more recent approach to evaluate the clinical outcome in breast cancer patients, the genome-wide expression pattern of thousands of genes can be simultaneously assayed by utilizing cDNA microarrays. Classification of tumors based on these gene expression patterns can then be used as a prognostic marker of the clinical outcome (Perou *et al.* 2000, Sørlie *et al.* 2001). For instance, a specific gene expression signature associated with poor prognosis was recently identified based on microarray data from 117 breast tumors (van't Veer *et al.* 2002).

2.3 Hereditary breast cancer predisposition

Breast cancer is recognized as the most prevalent malignancy among females worldwide (Parkin *et al.* 1993). The number of new cases has been increasing annually, and in 1999, altogether 3585 new breast cancer cases were reported in Finland (Finnish Cancer Registry 2002). However, mortality rates have been declining at the same time. The most prominent and therefore one of the best studied risk factors is a family history of breast cancer. Women who carry either a *BRCA1* or a *BRCA2* mutation have an estimated 60% lifetime risk of developing breast cancer (Easton *et al.* 1993, Ford *et al.* 1998). They also have a high lifetime risk of ovarian cancer, although for *BRCA2* carriers, the risk is somewhat lower. In addition to breast and ovarian cancer, an excess of prostate and colon cancers has been observed in families with *BRCA1* mutations (Ford *et al.* 1994). Men carrying a *BRCA2* mutation have an estimated 6% lifetime risk of breast cancer, and *BRCA2* mutations may also be associated with prostate and pancreatic cancer (Breast Cancer Linkage Consortium 1999). Furthermore, mutations in genes linked to certain inherited cancer-predisposing syndromes are known to increase the risk of breast cancer (Nathanson et al. 2001).

Besides family history, early age of menarche, nulliparity, late age of menopause and benign breast diseases are considered less powerful endogenous cancer risk factors. Of the exogenous risk factors, radiation exposure has clearly been shown to increase the cancer risk. Additional known exogenous risk factors are the use of oral contraceptives, a high-fat diet and high socio-economic status (Couch & Weber 1998 and refs. therein).

2.3.1 Beyond BRCA1 and BRCA2

Environmental factors, such as geographically limited exposure to carcinogens, cultural behavior patterns (e.g. age at first live birth) or socio-economic influences (e.g. dietary routine) undeniably play a role in familial clustering of breast cancer. However, approximately 7% of breast cancers are caused by the inheritance of a germline mutation in a cancer-predisposing gene (Claus *et al.* 1996). Mutations in the two most important, high-penetrance breast cancer susceptibility genes, *BRCA1* and *BRCA2* (Miki *et al.* 1994, Wooster *et al.* 1995), were found in a majority of breast cancer families with apparent autosomal dominant inheritance of susceptibility to both breast and ovarian cancer (Ford *et al.* 1998). Among these families, especially the proportion of *BRCA1* mutations was strikingly high (52%). However, later studies confirmed the observation that the breast cancer predisposition in a majority of families with less than six cases of female breast cancer and no ovarian cancer is not due to *BRCA1* or *BRCA2* mutations (Rebbeck *et al.* 1996, Schubert *et al.* 1997, Serova *et al.* 1997, Vehmanen *et al.* 1997a,b, Huusko *et al.* 1998). In Finland, most of the breast-ovarian cancer families were found to be *BRCA1* or *BRCA2* mutation-positive, while only 5-11% of the breast cancer families lacking ovarian cancer carried these mutations (Vehmanen *et al.* 1997a,b, Huusko *et al.* 1998, Vahteristo *et al.* 2001a). Additionally, germline mutations in the *TP53*, *ATM, PTEN, LKB1* and *AR* genes have been reported to increase the risk of breast cancer (Swift *et al.* 1987, Malkin *et al.* 1990, Srivastava *et al.* 1990, Wooster *et al.* 1992, Lobaccaro *et al.* 1993, Tsou *et al.* 1997, Boardman *et al.* 1998). The involvement of *TP53* and *ATM* will be discussed in more detail later (see chapters 2.5.1.1 and 2.5.1.2).

Evidently, a residual dominantly inherited risk for breast cancer in addition to the risk due to mutations in *BRCA1* and *BRCA2* does exist. In addition, there is a substantially increased recessively inherited risk associated with early-onset breast cancer (Cui *et al.* 2001). Multiple approaches are now being used to identify additional cancer susceptibility genes (Nathanson & Weber 2001). For example, genetic linkage studies are being performed on families with three or more cases of breast cancer. Positive linkage was observed at the chromosome region 8p12-p22 in two German breast cancer families (Seitz *et al.* 1997), but was not confirmed in a larger study (Rahman *et al.* 2000). In a study of Finnish, Swedish and Icelandic breast cancer families, another plausible breast cancer susceptibility locus at chromosome 13q21-q22 was identified (Kainu *et al.* 2000). Somatic deletions detected by comparative genomic hybridization (CGH) were used as the basis of a targeted linkage analysis of 77 families, resulting in a multi-point LOD score of 3.46. This locus (OMIM 605365) has been estimated to explain approximately two thirds of Finnish *BRCA1* and *BRCA2* mutation-negative breast cancer families. The results obtained in this study were also evaluated in 128 high-risk breast cancer families of Western European ancestry carrying no identified *BRCA1* or *BRCA2* mutations, but no evidence of linkage was found (Thompson *et al.* 2002). Thus, the authors of the latter study concluded that if a susceptibility gene does exist at this locus, its contribution to breast cancer susceptibility is likely to be very small or at least geographically limited. However, the characteristic clustering of genetic disease alleles in Finland (Peltonen *et al.* 1999) and the different ascertainment criteria of the study cohort might explain the

contradictory linkage results and the variation in the observed *BRCA1* and *BRCA2* mutation frequencies in different populations.

As genetic linkage studies have had limited success in identifying new breast cancer susceptibility genes, genome-wide association studies utilizing single nucleotide polymorphisms (SNPs) could provide an efficient tool suitable for automated high-throughput genotyping and identification of disease-associated haplotypes (Irizarry *et al.* 2000, International SNP Map Working Group 2001). Classification of breast cancer families according to the gene expression data from cDNA microarrays (Hedenfalk *et al.* 2001) and screening for altered gene products using oligonucleotide-based exon-specific arrays (Shoemaker *et al.* 2001) might also prove invaluable in the search for new susceptibility genes.

2.3.2 Low penetrance candidate genes on the basis of biological plausibility

Sequence variants or polymorphisms in low-penetrance genes might be associated with a slightly elevated risk for breast cancer. However, due to their frequent occurrence, these alterations might confer a much higher attributable cancer risk in the general population than rare mutations in high-penetrance genes (Nathanson & Weber 2001). Based on the biological plausibility, candidate low-penetrance genes are usually chosen from among the genes in which even a subtle change could have an effect on the biochemical pathways that influence carcinogenesis. For example, the genes involved in carcinogen and steroid hormone metabolism, DNA damage repair and immune surveillance have been hypothesized as candidates for low-penetrance cancer susceptibility genes (Nathanson & Weber 2001). Due to the rare appearance of low-penetrance alleles in cancer families appropriate for genetic linkage studies, either population-based case-control studies or evaluation of low-penetrance genes as modifiers of high-penetrance genes are currently the two most suitable approaches. Based on case-control studies, certain polymorphisms in the *CYP19*, *GSTM1*, *GSTP1* and *TP53* genes seem to be candidates for low-penetrance genes (Dunning *et al.* 1999). Association studies have demonstrated that women carrying a *BRCA1* mutation and an altered form of either *HRAS* or the androgen receptor gene have an increased cancer risk compared to those carrying only the *BRCA1* mutation (Phelan *et al.* 1996, Rebbeck *et al.* 1999). A single nucleotide polymorphism in the *RAD51* gene has also been reported to increase the cancer risk in *BRCA2* carriers (Levy-Lahad *et al.* 2001). Both approaches, however, have their limitations. Case-control studies are generally time-consuming and expensive, and the association studies of modifier genes are often underpowered, lacking an appropriate control population (Nathanson *et al.* 2001, Nathanson & Weber 2001).

2.4 Cellular pathways maintaining genomic integrity

2.4.1 DNA damage repair pathways

Deficiencies in DNA damage signaling and repair pathways are fundamental to the etiology of most human cancers. Throughout its biological life, DNA is exposed to a variety of different damaging agents, which can be of either exogenous or endogenous origin. No single repair process could manage with the great variety of genetic lesions, and multiple, partly overlapping damage repair pathways are therefore required in mammals. The major mechanisms of DNA repair include excision, recombinational and mismatch repair (Figure 1) (Hoeijmakers 2001). Many human syndromes predisposing the patient to cancer are due to defects in these repair pathways (Table 1).

Table 1. Aberrations of the components in cell cycle checkpoint and DNA repair in human tumors.

Gene	Defect	Hereditary syndrome	Cancer
ATM	DSB	Ataxia-telangiectasia	lymphoma, leukemia, breast
MRE11A	DSB	AT-like disorder	lymphoma
NBS1	DSB	Nijmegen breakage syndrome	lymphoma
BRCA1	HR	Familial breast cancer 1	breast, ovarian, prostate, colon
BRCA2	HR	Familial breast cancer 2	breast (female/male), ovary, prostate, pancreas
CHK1	CC	NR	colorectal and endometrial cancer
CHK2	CC	Li-Fraumeni syndrome	breast, lung, colon, urinary bladder, testis
p53	CC	Li-Fraumeni syndrome	sarcoma, breast, brain, leukemia,
RECQL2	HR?	Werner syndrome	various cancers
RECQL3	HR?	Bloom syndrome	leukemia, lymphoma
RECQL4	HR?	Rothmund-Thomson syndrome	osteosarcoma
MSH2	MMR	HNPCC	colon, rectum, gastric, endometrium, ovarian,
MLH1		"	urinary organs
PMS2		"	
MSH6		"	
MLH3		"	
p16	CC	Familial melanoma	melanoma, pancreas
RB	CC	Familial retinoblastoma	retinoblastoma, osteosarcoma
CSA, CSB	TCR	Cocayne's syndrome	Skin
XPA-XPG	NER	Xeroderma pigmentosum	skin

Abbreviations: CC, cell cycle control; DSB, double-strand break repair; HR, homologous recombination repair; MMR, mismatch repair; NER, nucleotide excision break repair; NR, not reported; TCR, transcription coupled repair. Adapted from Bartek & Lukas (2001), Hoeijmakers (2001), Svejstrup JQ (2002).

Two basic types of excision repair, nucleotide excision repair (NER) and base excision repair (BER), have been described both in bacteria and in mammals (reviewed in Hoeijmakers 1993a,b, Wood 1997). NER acts on a variety of helix-distorting DNA

lesions mostly caused by exogenous sources interfering with base pairing. The most important function of NER in humans is to remove ultra violet (UV)-induced damage from DNA (e.g. pyrimidine dimers). Defects in NER cause an autosomal recessive disease called xeroderma pigmentosum (XP), which makes the patients extremely prone to develop sun-induced skin malignancies. XP is due to mutations in seven different genes, *XPA*, *XPB*, *XPC*, *XPD*, *XPE*, *XPF* and *XPG*, all of which function in the NER pathway (Hoeijmakers 2001).

Eukaryotic NER includes two major branches, transcription-coupled repair (TCR) and global genome repair (GGR) (de Laat *et al.* 1999, Tornaletti & Hanawalt 1999). GGR is a slow random process of inspecting the entire genome for injuries, while TCR is highly specific and efficient and concentrates on damage-blocking RNA polymerase II. The two mechanisms differ in substrate specificity and recognition. In GGR, the XPC-HR23B complex recognizes damage located in nontranscribed regions (Sugasawa *et al*. 2001), whereas the arrest of RNA polymerase II (RNAPII) serves as the recognition signal in TCR. The molecular mechanism of RNAPII displacement is currently unclear, but essential factors, such as the Cocayne's syndrome proteins CSA, CSB, XPA-binding protein 2 (XAB2), TFIIH and XPG (Svejstrup 2002), have been identified to function in TCR. Subsequently, both in GGR and TCR, an open unwound structure forms around the lesion. This creates specific cutting sites for XPG and ERCC1-XPF nucleases, and the resulting gap is filled in by PCNA-dependent polymerase and sealed by DNA ligase (Wood 1997, de Laat *et al.* 1999).

BER removes small chemical alterations of bases, focusing mainly on modifications due to endogenous cellular metabolism (e.g. reactive oxygen species and byproducts of hydrolysis, methylation and deamination) (Lindahl & Wood 1999, Hoeijmakers 2001). In BER, a lesion-specific glycosylase removes the modified bases from DNA, resulting in the generation of an apurinic or apyrimidinic (AP) site. The AP endonuclease then cuts the sugar-phosphate backbone at the position of the missing base. Exonucleases further remove a few nucleotides from the DNA, and the remaining gap is filled by resynthesis and sealed by DNA ligase 3 (Lindahl & Wood 1999). Similarly to NER, there is substantial evidence that TCR can also occur through the BER pathway (Cooper *et al.* 1997, Nouspikel *et al*. 1997, Le Page *et al*. 2000).

For the cell, double-strand breaks (DSB) are probably the most deleterious form of DNA damage and may arise from ionizing radiation (IR), X-rays, free radicals, chemicals, or during replication of single-strand breaks (SSB) (Khanna & Jackson 2001). A single DSB is sufficient to kill a cell if it inactivates an essential gene or triggers apoptosis (Rich *et al.* 2000). There are two distinct and complementary mechanisms for DSB repair: homologous recombination (HR) and non-homologous end-joining (NHEJ) (Haber 2000, Karran 2000). When an intact DNA copy is available, HR is preferred. Otherwise, cells utilize the more error-prone NHEJ. It has been proposed that Ku70 acts as a switch between the two DSB pathways (Goedecke *et al.* 1999). When present, Ku70 destines DSB for NHEJ by binding to DNA ends and attracting other factors, including MRE11. However, the absence of Ku70 allows participation of DNA ends together with MRE11 in the meiotic HR pathway.

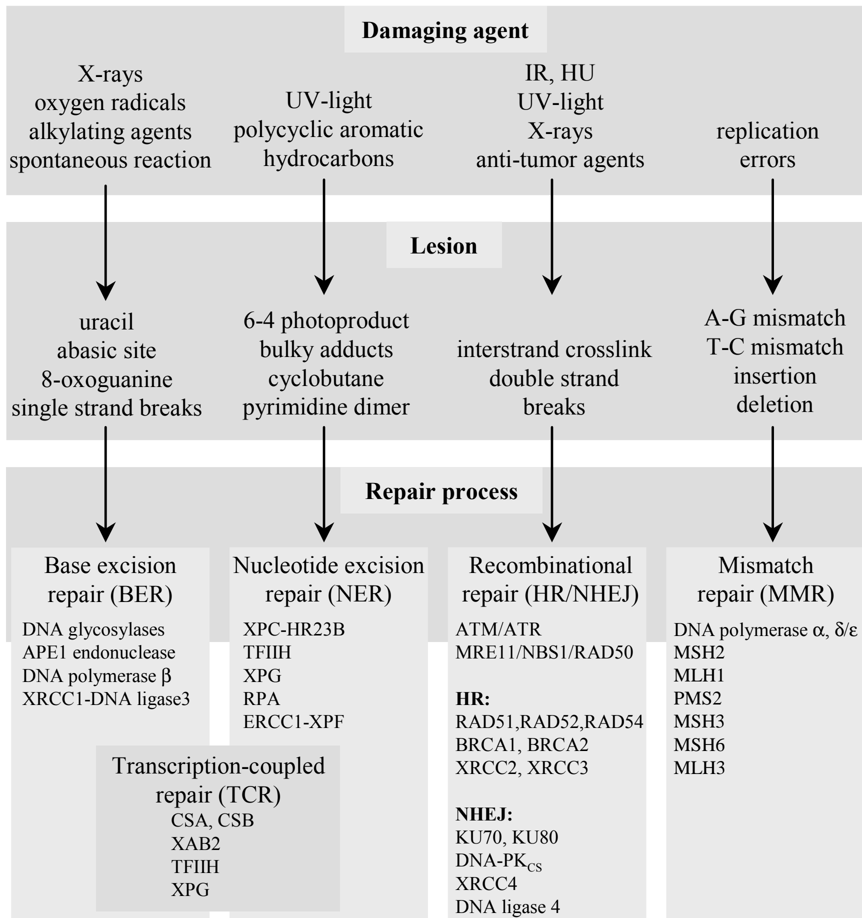

Fig. 1. DNA-damaging agents (top), examples of DNA lesions (middle), and the relevant repair mechanisms (bottom). The essential genes involved in each DNA repair pathway are shown below the corresponding titles. HR, homologous recombination; NHEJ non-homologous end-joining; TCR, transcription-coupled repair. Adapted from Hoeijmakers (2001), Khanna & Jackson (2001), Svejstrup (2002).

In HR, the DNA ends are first resected in the 5’ to 3’ direction by the exonuclease activity of the RAD50/MRE11/NBS1 complex (Paull & Gellert 1998). Assisted by several proteins facilitating the identification and correct positioning with the homologous sister chromatid sequence, the 3’ single-stranded tails invade the DNA double helix of the intact molecule, which is used as a template for DNA polymerase. Following branch migration and ligation, Holliday junctions are resolved by resolvases to yield two intact DNA molecules (Khanna & Jackson 2001). On the contrary, NHEJ does not require an undamaged template: here, the two DNA ends are simply attached together

using the end-binding Ku70/80 complex and DNA-PK$_{CS}$, followed by ligation by XRCC4 (Haber 2000, Hoeijmakers 2001, Khanna & Jackson 2001). Mutations in many of the genes involved in DSB detection and repair give rise to many cancer-predisposing syndromes (Table 1).

Mismatch repair (MMR) removes both nucleotides mispaired by DNA polymerases and insertion/deletion loops, which are due to slippage during replication of repetitive sequences or formed during recombination (Harfe & Jinks-Robertson 2000). Initially, the heterodimeric MSH complex recognizes the nucleotide mismatch, subsequently followed by interaction with MLH1/PMS2 and MLH1/MLH3 complexes. Several proteins participate in process of the nucleotide excision and resynthesis. Tumor cells deficient in mismatch repair have much higher mutation frequencies than normal cells (Parsons *et al.* 1993, Bhattacharyya *et al.* 1994). In humans, the mechanism involves at least six genes *MSH2*, *MLH1*, *PMS2*, *MSH3*, *MSH6* and *MLH3*. The defects in these genes (excluding *MSH3*) result in hereditary nonpolyposis colon cancer (HNPCC) (Hoeijmakers 2001, Peltomäki 2001).

Traditionally, the DNA repair mechanisms have been regarded as separate processes. However, since the different replication complexes share many of the participating proteins, it is possible that these partly similar processes should be regarded as temporary associations of DNA interacting proteins rather than as rigid complexes where the participating proteins are devoted solely to one mission (Cleaver *et al.* 2001). The various associations of these complexes would occur according to the specific challenges set by DNA damage. An example of such complex is the BRCA1-associated genome surveillance complex (BASC), which contains many known DNA polymerases, repair enzymes and recombination proteins (Wang *et al.* 2000) (Table 2). All of these proteins possess the ability to bind abnormal DNA structures and might therefore act as sensors for these aberrations (Uchiumi *et al.* 1996, Alani *et al.* 1997, Bennett *et al.* 1999, Marsischky *et al.* 1999).

It has been proposed that BRCA1 has a central role in BASC (Table 2), acting as a scaffold that both organizes and coordinates the multiple activities required to maintain genomic integrity (Wang *et al.* 2000). BRCA1 has two C-terminal BRCT (BRCA1 carboxy-terminal repeat) domains, which are important for protein-protein interactions and have been found in many proteins to be linked to DNA damage response checkpoints (Koonin *et al.* 1996, Bork *et al.* 1997, Zhang *et al.* 1998a). BRCA1 has also been found to participate in other regulatory complexes, such as the RNA polymerase II holoenzyme (Scully *et al.* 1997) and the Swi/Snf chromatin remodeling complexes (Bochar *et al.* 2000), connecting BRCA1 to transcription regulation and providing a link to IR-induced transcription-coupled repair. BRCA2, on the other hand, regulates both the intracellular localization and the DNA-binding ability of RAD51, which is an essential protein in homologous recombination and DNA repair (Davies *et al.* 2001).

Table 2. Suggested roles of known proteins and protein complexes in the BRCA1-associated genome surveillance complex (BASC).

Protein or subcomplex	DNA damage sensor	DNA repair mechanism
BRCA1	scaffold protein to organize different DNA damage sensor proteins	DSB repair
ATM	double-strand break detection	signal transduction of DSB response
MRE11/RAD50/NBS1	double-strand break detection	DSB repair
MSH2/MSH6	base-pair mismatches Holliday junctions	MMR
PMS2/MLH1	base-pair mismatches	MMR
RECQL3	abnormal double-stranded structures during replication abnormal telomere repeat sequences	HR?
RF-C complex	abnormal template-primer junctions	NER

2.4.2 Apoptotic pathways

The efficiency of the DNA repair mechanisms, cell type, the oncogenic composition of the cell, extracellular signals, the intensity of the stress conditions, the level of p53 expression and its interactions with other proteins all have an effect on the cell's response to the existing damage (reviewed in Vogt Sionov & Haupt 1999). Should the damage be irreparable, a defective cell can be sacrificed for the benefit of the organism. Two main apoptotic pathways have been described in mammalian cells, namely the death-receptor pathway and the mitochondrial pathway (Hengartner 2000). Members of the death-receptor superfamily, including CD95, trigger the death-receptor pathway. After DNA damage, p53 is essential for transcriptional upregulation of CD95 (Müller *et al.* 1997, Müller *et al.* 1998). In general, signaling of apoptosis by members of the death receptor family includes ligand binding, receptor trimerization and death inducing signaling complex (DISC) formation, recruitment of multiple procaspase-8 molecules, and subsequent autocatalytic cleavage of the procaspase. Active caspase-8 then activates other downstream caspases that cleave cellular substrates, leading to cell destruction (Hengartner 2000).

The mitochondrial pathway, on the other hand, is utilized in response to extracellular signals and internal insults such as DNA damage (Kroemer & Reed 2000, Rich *et al.* 2000). Apart from its essential role in regulating cell growth, p53 also participates in this cell death pathway. Its function leads to elevated BAX-levels (Miyashita & Reed 1995),

whereas the expression of BCL2 is downregulated through abolished promoter function (Budhram-Mahadeo *et al.* 1999). Pro- and anti-apoptotic BCL2 family members meet at the surface of mitochondria, where they compete to regulate cytochrome *c* release (Gross *et al.* 1999). Together with Apaf-1 and procaspase-9 and possible additional proteins, cytochrome *c* participates in apoptosome formation (Acehan *et al.* 2002). The two pathways come together at the level of caspase-3 activation, and the following multiple downstream pathways finally result in organized destruction and removal of the destined cell (Hengartner 2000).

2.5 DNA double-strand break signaling and its relation to cell cycle control

The DNA DSB damage response pathway provides a mechanism for transducing a signal from a sensor, which recognizes the damage, through a transduction cascade to a series of downstream effector molecules. Depending on the severity of the damage, cells may either choose to arrest the cell cycle until the damage is repaired, or if the damage is irreparable, proceed to apoptosis. A schematic presentation of the mammalian DSB signaling pathway is shown in Figure 2.

The two main sensor molecules, ATM and ATR, act in parallel branches at the front line of the DNA damage response pathway. They respond primarily to different types of DNA damage (Gatei *et al.* 2001). ATM reacts mainly to the DNA-damaging agents that cause DSBs, such as IR, and its downstream targets include CHK2, BRCA1 and p53 (Banin *et al.* 1998, Canman *et al.* 1998, Matsuoka *et al.* 1998, Cortez *et al.* 1999, Matsuoka *et al.* 2000). On the other hand, ATR, along with ATRIP (ATR-interacting protein), responds primarily to UV and hydroxyurea (HU)-induced damage, which may potentially interfere with DNA replication (Cortez *et al.* 2001). ATR regulates CHK1 and BRCA1 (Chen 2000, Liu *et al.* 2000), but phosphorylation of p53 is also possible (Tibbetts *et al.* 1999). Due to ATM and ATR, BRCA1 becomes phosphorylated not only at overlapping but also at distinct residues, depending on the type of the DNA lesion (Gatei *et al.* 2001). Furthermore, the two pathways overlap and often cooperate with each other to ensure efficient repair without delay and to maintain genomic integrity. When one pathway is genetically compromised, they can also function redundantly, although probably less efficiently and with different kinetics (Liu *et al.* 2000).

Cell cycle arrest is controlled at specific checkpoints. At the G1 and S checkpoints, the quality of the DNA about to be replicated is ensured, whereas at the G2-M, the segregation of damaged chromosomes is prevented. At G1, as a consequence of DNA damage, activated ATM phosphorylates p53 on Ser15, CHK2 on Thr68, and MDM2 on Ser395 (Banin *et al.* 1998, Canman *et al.* 1998, Ahn *et al.* 2000, Matsuoka *et al.* 2000, Melchionna *et al.* 2000, Maya *et al.* 2001). Subsequently, activated CHK2 phosphorylates p53 on Ser20 (Hirao *et al.* 2000, Shieh *et al.* 2000). Together, these phosphorylations interfere with p53 binding to MDM2, leading to stabilization and activation of p53 (Chehab *et al.* 2000). The increased level of p53 transcriptionally induces p21 (also known as CDKN1A and WAF1), which inhibits CDK2-cyclin E causing the cell cycle arrest (Kastan & Lim 2000).

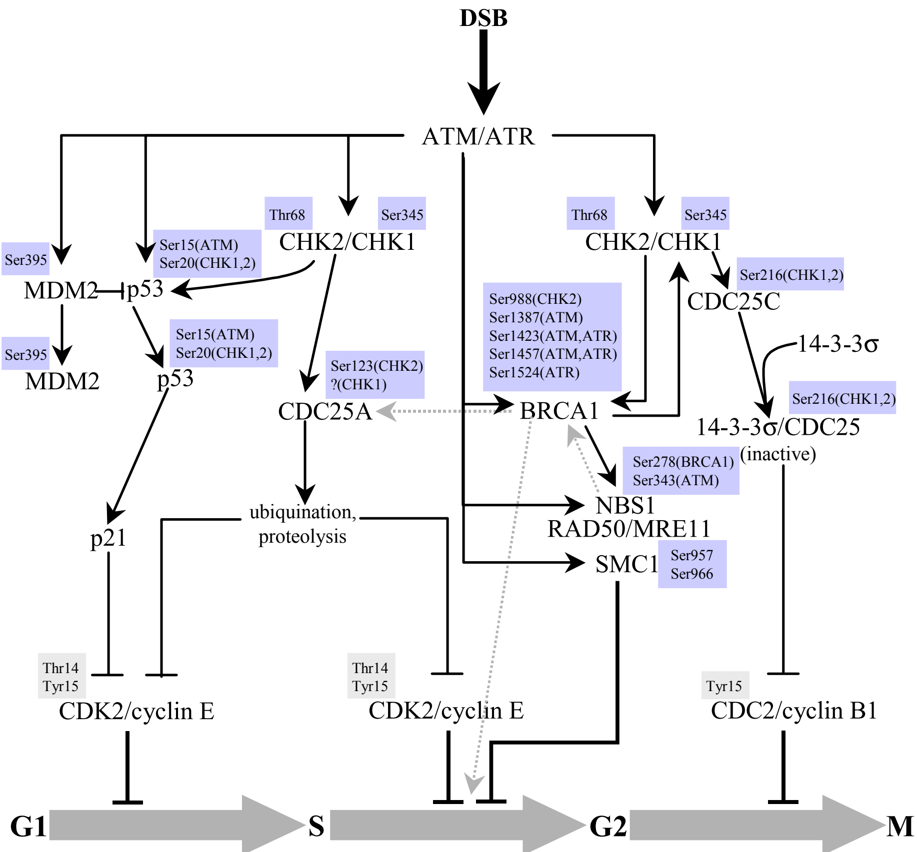

Fig. 2. A schematic representation of the DNA DSB signaling pathway. Phosphorylation sites, when known, are indicated next to each protein. The sites with a lighter background are inhibitory residues, which have to be removed by an upstream effector. The dashed arrows represent unidentified, putative parallel or converging pathways influencing S-phase arrest. Modified from Vogt Sionov & Haupt (1999), Kastan & Lim (2000), Bartek & Lukas (2001), Futaki & Liu (2001), Khanna & Jackson (2001), Taylor & Stark (2001), Kim *et al.* (2002), Yazdi *et al*. (2002).

The defective S-phase checkpoint is defined by a phenomenon of persistent DNA synthesis after IR (also called radioresistant DNA synthesis). In contrast to G1 and G2, the S phase can only be delayed, but never permanently blocked. This delay seems to be independent of the p53 and p21 functions (Bartek & Lukas 2001). Instead, a pathway operating through ATM/ATR, CHK2/CHK1 and CDC25A prevents the removal of inhibitory phosphorylations of CDK2 (Mailand *et al.* 2000, Costanzo *et al.* 2000, Falck *et*

al. 2001b). Besides its importance in the S-phase response, this pathway also provides an alternative route for generating rapid arrest in G1. Activated ATM phosphorylates CHK2 (Matsuoka *et al.* 2000, Melchionna *et al.* 2000), which then targets CDC25A phosphatase for degradation by phosphorylation it on Ser123 (Falck *et al.* 2001b), and thus prevents the activation of CDK2. In a parallel S-phase checkpoint pathway (Falck *et al.* 2002), ATM phosphorylates NBS1 on Ser343, which is an event required for activation of NBS1/MRE11/RAD50 complex (Lim *et al.* 2000, Zhao *et al.* 2000). Concurrently, ATM phosphorylates SMC1 on Ser957 and Ser966 in an NBS1-dependent manner, and these phosphorylations are required for S-phase checkpoint activation (Kim *et al.* 2002, Yazdi *et al.* 2002).

In G2, ATM and ATR activate the checkpoint kinases CHK2 and CHK1, respectively (Matsuoka *et al.* 2000, Zhao & Piwnica-Worms 2001), but also BRCA1 is essential for CHK1 activation (Yarden *et al.* 2002). Subsequently, CHK2 and CHK1 phosphorylate the protein phosphatase CDC25C on Ser216, which leads to its inactivation and binding by the 14-3-3σ protein (Peng *et al.* 1997, Sanchez *et al.* 1997, Matsuoka *et al.* 1998). This binding prevents the removal of an inhibitory phosphate group on Thr14 and Tyr15 of CDC2 and thus also the cell's entry to mitosis (Lee *et al.* 1992, Sebastian *et al.* 1993). The G2 checkpoint is also controlled by p53 through transcriptional repression of *Cdc2* and *cyclin B1*. Several other transcriptional targets of p53 can inhibit CDC2: p21 inhibits CDC2 directly, 14-3-3σ anchors CDC2 in the cytoplasm, where it cannot induce mitosis, and GADD45 dissociates CDC2 from Cyclin B1 (Taylor & Stark 2001).

2.5.1 Double-strand break signaling and DNA repair genes associated with inherited cancer syndromes

2.5.1.1 Germline TP53 and CHK2 mutations in Li-Fraumeni syndrome

Li-Fraumeni syndrome (LFS) is a rare familial multicancer syndrome involving sarcomas, breast cancer, brain tumors, leukemia, and adrenocortical tumors (Li & Fraumeni 1969, Li *et al.* 1988). The criteria for classic LFS are: a proband with sarcoma before the age of 45, a first-degree relative with any cancer by age 45, and another first- or second-degree relative with any cancer before age 45 or sarcoma at any age (Li *et al.* 1988). In many LFS families, affected members carry a germline *TP53* mutation (Malkin *et al.* 1990, Varley *et al.* 1997). Having discovered *TP53* mutations in families with a less severe cancer phenotype, Birch *et al.* (1994) presented criteria for a somewhat milder form of LFS, namely the Li-Fraumeni-like syndrome (LFL). In this case, a family would exhibit a proband with any childhood cancer or sarcoma, brain tumor, or adrenocortical carcinoma diagnosed before age 45, with one first- or second-degree relative with LFS-type cancer at any age, and one first- or second-degree relative with any cancer at age 60.

The mutation distribution along the protein-encoding region of the *TP53* gene was investigated by Soussi & Béroud (2001). By reviewing the recent studies, they

demonstrated that the mutations are not only concentrated to the highly conserved regions between the exons 5 and 8, but a considerably high proportion are also seen outside this section of the gene. *TP53* differs from the other tumor suppressor genes (e.g. *APC* and *BRCA1*) showing a high prevalence of missense mutations (Olivier & Hainaut 2001). In breast tumors, *TP53* is the most commonly altered gene with a frequency ranging from 12-60% (Olivier & Hainaut 2001). Breast cancer is also the most frequent type of cancer in patients with inherited *TP53* mutations, suggesting that a constitutive *TP53* mutation could also predispose to early onset breast cancer. Therefore, the role of *TP53* as a breast cancer predisposing gene, even without the LFL/LFS features, has been studied in considerable detail. When the *TP53* mutation pattern in families showing excessive cases of breast cancer was compared to all germline mutations, an absence of transversions affecting G bases and an excess of mutations in A:T base pairs was observed (Olivier & Hainaut 2001).

In a fraction of the LFS families lacking alterations in *TP53*, *CHK2* mutations have been found to explain the necessary disease predisposition (Bell *et al.* 1999, Vahteristo *et al.* 2001b). Due to its essential functions in DSB signaling and close intractions with p53 and BRCA1 (see chapter 2.5), defects in *CHK2* can also be hypothesized to have breast cancer predisposing effects similar to those of the mutations in *TP53* and *BRCA1*.

2.5.1.2 Germline ATM mutations in ataxia-telangiectasia

Ataxia-telangiectasia (AT) is a highly pleiotropic, recessive neurodegenerative disorder resulting from germline mutations in the *ATM* gene (Savitsky *et al.* 1995a). AT is characterized by progressive cerebellar ataxia, oculocutaneous telangiectasias, premature aging, hypogonadism, sensitivity to ionizing radiation and immunodeficiency. Furthermore, due to *ATM* involvement in DSB repair, defects in ATM protein function cause genetic instability and, consequently, an increased risk of cancer. AT patients are especially prone to develop lymphatic and leukemic malignancies, but also breast cancer. Cells derived from AT patients are hypersensitive to DSB-inducing agents (Gatti 1998).

The *ATM* gene located at 11q22.3 is composed of 66 exons and encodes a 13-kb mRNA (Uziel *et al.* 1996). ATM is a 370 kDa phosphoprotein, which is ubiquitously expressed. ATM is localized mainly in the nuclei of fibroblasts or lymphoid cells, but also in the cytoplasm, where it appears to be associated with vesicular structures through its interaction with β-adaptin (Chen and Lee 1996, Brown *et al.* 1997, Watters *et al.* 1997, Lim *et al.* 1998). In Purkinje cells and other neurons, however, ATM is predominantly cytoplasmic, and its absence leads to lysosome accumulation (Oka & Takashima 1998, Barlow *et al.* 2000). It belongs to a family of large proteins identified in various organisms, all of which share a highly conserved carboxy-terminal region showing significant sequence homology to the catalytic domain of phosphatidyl inositol-3 kinases (Savitsky *et al.* 1995b).

In addition to its role in the cellular response to DNA damage (see chapter 2.5), ATM activates a separate radiation signal transduction pathway through the stress-activated protein kinase by interacting with ABL (Baskaran *et al.* 1997, Shafman *et al.* 1997)

(Figure 3). Cytoplasmic ATM may also have a role in vesicle and protein transport (Watters *et al.* 1997, Lim *et al.* 1998) and in protection against endogenous oxidative damage (Barlow *et al*. 1999). Based on observations in mice, Atm links to the mitochondrial apoptotic pathway due to its association with Bax (Chong *et al.* 2000) and is specifically cleaved by caspase-3 in cells induced to undergo apoptosis (Smith *et al.* 1999). This interferes with the ability of ATM to phosphorylate p53, but does not have an effect on its DNA-binding ability. Since ATM is able to bind DNA ends *in vitro*, it is possible that cleaved ATM binds to the genomic DNA DSBs generated during the apoptotic process, preventing their repair (Smith *et al.* 1999). In the central nervous system of mice, Atm participates in genotoxic damage-induced apoptosis, aiming to eliminate genetically damaged neurons (Herzog *et al.* 1998). ATM also seems to have a role in telomere maintenance and replication (Hande *et al.* 2001).

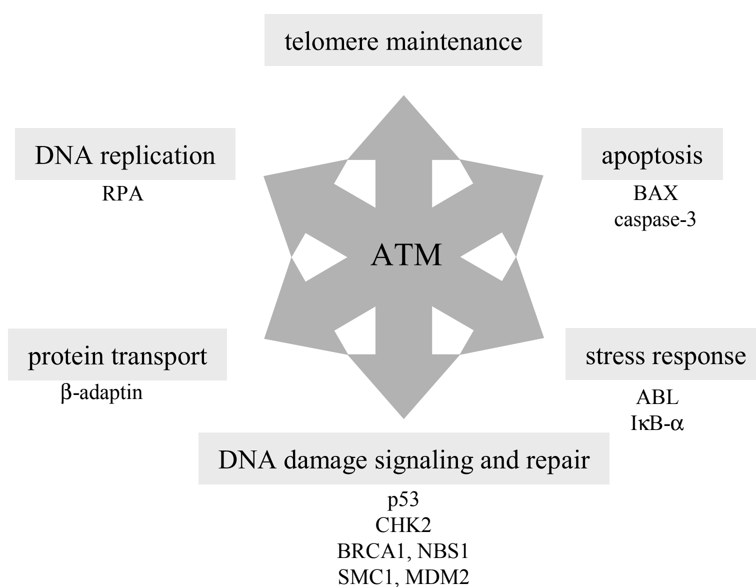

Fig. 3. The many functions of ATM. Some of the key interacting proteins are shown under the title of the involved function. According to Ángele & Hall (2000), Kastan & Lim (2000).

The observation of intermediate radiosensitivity in cells from AT heterozygotes by Chen *et al.* (1978) first suggested that these individuals might have an elevated cancer risk. Several years later, it was proposed that chromosomal radiosensitivity could be a marker of a low-penetrance cancer-predisposing gene (Scott *et al.* 1994, Scott *et al.* 1999). To validate this hypothesis, the same group demonstrated the heritability of chromosomal radiosensitivity of G2 lymphocytes in families with breast cancer (Roberts *et al.* 1999). Segregation analysis suggested that a single gene could account for as much as 82% of the variance in radiosensitivity observed between the family members, but the data could also be explained by using an alternative model including another, even rarer

gene defect. In addition, increased sensitivity to radiation-induced oncogenesis has recently been demonstrated in murine models carrying heterozygous *Atm* mutations (Smilenov *et al.* 2001, Weil *et al.* 2001). Despite this observation, radiation therapy still appears to be beneficial for carriers of *ATM* germline mutations (Su & Swift 2001)

The estimated frequency of *ATM* germline mutation carriers in Finland is one in 280 (Olsen *et al.* 2001). In epidemiological studies, the female relatives of AT patients, especially mothers, have been demonstrated to have an increased risk to develop breast cancer, with an estimated relative risk of 3.9 (Swift *et al.* 1987, Pippard *et al.* 1988, Swift *et al.* 1991, Janin *et al.* 1999, Inskip *et al.* 1999, Olsen *et al.* 2001). The risk of any other cancers in obligate mutation carriers is not increased in a similar manner (Geoffroy-Perez *et al.* 2001). A clear discrepancy remains between the epidemiological data and the observed low frequency of *ATM* germline mutations in breast cancer patients (Athma *et al.* 1996, Vorechovský *et al.* 1996, FitzGerald *et al.* 1997, Bebb *et al.* 1999, Izatt *et al.* 1999, Laake *et al.* 2000b). Interestingly, a high percentage (8.5%) of *ATM* truncating mutations was demonstrated among patients with sporadic breast cancer (Broeks *et al.* 2000). All the studied patients had been exposed to radiation therapy, suggesting that radiation might, indeed, induce the development of breast cancer. On the contrary, truncating mutations were not found in a cohort of breast cancer patients with contralateral tumors who had received radiation therapy for their first tumor (Shafman *et al.* 2000). Thus, the role of radiation as a trigger of tumorigenesis still warrants further investigation. Furthermore, both studies utilized methods that were incapable of detecting missense mutations.

It has been hypothesized that there are two different populations of heterozygous *ATM* carriers (Meyn 1999, Khanna 2000), having one allele carrying either a truncating or a missense mutation in combination with a normal *ATM* allele. In the first group, a truncating mutation acts effectively as a null allele, producing an unstable protein, which is present in the cell in very low amounts. Therefore, carriers with a truncating mutation would have a nearly normal phenotype. Instead, in carriers with a missense mutation, a stable but functionally abnormal protein would be present at normal intracellular levels. These abnormal polypeptides would compete with the normal protein in complex formation, thus interfering with their essential cellular functions and contributing to an increased cancer risk. Thus, to elucidate the significance of the proposed hypothesis, it would be important to determine the frequency of *ATM* missense variants in the general population in greater detail. It is possible that the reasons why these studies have failed to detect the expected number of cancer-related *ATM* mutations is due to the choice of technique used in mutation screening as well as in the compositions of the study and control populations (reviewed in Ángele & Hall 2000).

2.5.1.3 Germline mutations of MRE11A in ataxia-telangiectasia-like disorder and NBS1 in Nijmegen breakage syndrome

Apart from AT, there are two other phenotypically very similar chromosome breakage syndromes. Germline mutations in *MRE11A* give rise to an AT-like disorder (ATLD)

(Stewart *et al.* 1999), whereas defects in *NBS1* (nibrin, p95) result in Nijmegen breakage syndrome (NBS) (Carney *et al.* 1998, Varon *et al.* 1998). The clinical features of these three syndromes overlap, sharing the traits of cancer predisposition, immunodeficiency, hypersensitivity to radiation as well as chromosomal instability (Hoeijmakers 2001). Additionally, cells derived from affected individuals show very similar phenotypes (Petrini 2000). This can be explained by the interacting roles of these three proteins in the DSB pathway and S-phase checkpoint control (Figure 2). MRE11, RAD50 and NBS1 form a nuclear complex, which includes a manganese-dependent single-stranded DNA endonuclease and 3' to 5' exonuclease activities (Carney *et al.* 1998, Trujillo *et al.* 1998). Along with BRCA1, ATM, and many other proteins, the MRE11/RAD50/NBS1 complex is a member of BASC, which has been suggested to serve as a sensor of DNA damage and a regulator of the repair process (Wang *et al.* 2000) (see also 2.4.1). Furthermore, ATM phosphorylates NBS1 on several residues in response to DNA damage (Lim *et al.* 2000, Wu *et al.* 2000, Zhao *et al.* 2000), and a functional MRE11/RAD50/NBS1 complex is required for CHK2 activation (Buscemi *et al.* 2001). This links the MRE11/RAD50/NBS1 complex to DNA damage recognition and also provides a plausible explanation for the phenotypic similarities between these three chromosome breakage syndromes.

2.6 Somatically occurring alterations in breast cancer

As already discussed in chapter 2.1, cancer results from a series of genetic alterations leading to progressive disordering of the important regulatory cellular mechanisms (Fearon & Vogelstein 1990). A small fraction of breast cancers show inherited mutations in certain susceptibility genes, which thus cause the carrier to have a higher risk of developing breast cancer. For cancer to arise, however, several additional somatic mutations are required. Contrary to hereditary cancer, most tumors are devoid of inherited germline mutations. Despite this fundamental difference in molecular genetics between familial and sporadic tumors, many of the same genes are altered in both forms of the disease. For instance, the TSGs modified in sporadic tumors are also good candidates for susceptibility loci in corresponding familial disease. Moreover, studies of the somatic alterations (e.g. point mutations and other more extensive genetic rearrangements, such as insertions, deletions and amplifications, as well as aberrant DNA methylation) provide clues to the mechanisms that result in genomic instability, which is inherent in cancer cells (Couch & Weber 1998 and refs. therein). Indeed, it has been demonstrated that DSB-initiated chromosomal instability is the major driving force of breast cancer progression. Furthermore, a specific methylator phenotype possibly resulting in MMR deficiency has been observed in colorectal cancer (Toyota *et al.* 1999). It might be due to the genetic heterogeneity resulting from these defects that different breast tumors also present such a great variety of molecular profiles (Shen *et al.* 2000).

2.6.1 Loss of heterozygosity and comparative genomic hybridization studies in breast cancer

According to Knudson's 'two-hit' hypothesis (Knudson 1971), inactivation of both alleles of a TSG is required for cancer formation. In the hereditary form of cancer, the other allele has an inherited mutation derived from the affected parent, whereas the other allele is mutated somatically. In sporadic cancer, a somatic mutation targeting each allele is required to completely inactivate a TSG. Inactivating mutations include point mutations, loss of chromosomal material, gene conversion, or mitotic recombination or deletion (Knudson 1978, Cavenee *et al.* 1983). Therefore, chromosomal regions that frequently exhibit allelic losses are expected to harbor putative tumor suppressor genes (Sato *et al.* 1990). LOH and CGH analysis are currently the two methods most frequently used to detect chromosomal losses.

Traditional LOH analysis is a PCR-based method in which paired blood and tumor samples are screened with polymorphic microsatellite markers spaced across the region of interest. The possible allelic loss at each marker locus can be detected in tumor tissue compared to normal tissue of the same individual. CGH has been designed to detect amplified and deleted stretches of DNA in tumors (Kallioniemi *et al.* 1992). This technique has been recently used to identify the loci for the Peutz-Jehgers cancer syndrome and the putative new breast cancer susceptibility locus (Hemminki *et al.* 1997, Hemminki *et al.* 1998, Kainu *et al.* 2000). In principle, CGH provides the means to screen the entire genome for chromosomal imbalances in a single experiment. A mix of differently labeled DNAs from normal and tumor cells is used in competitive fluorescence *in situ* hybridization. The smallest alteration visible in standard CGH is 5-10 Mb, but by using high-density oligonucleotide arrays instead of metaphase chromosomes, CGH analysis can be significantly improved (Monni *et al.* 2001). Furthermore, in combination with microarray based expression analysis, CGH provides a powerful approach to identify the putative targets of gene amplification (Monni *et al.* 2001).

LOH in breast tumors has been observed in multiple chromosomal regions, such as the chromosomes 1p, 1q, 3p, 6q, 7q, 8p, 9p, 11q, 13q, 16q, 17p, 18q and 22q (Bièche & Lidereau 1995, Kerangueven *et al.* 1997a, O'Connell *et al.* 1998, Shen *et al.* 2000). By utilizing CGH, the most frequently deleted regions in breast cancer cell lines were found in the chromosomes 1p, 4p, 8p, 10q, 11q, 18p, 18q, 19p, Xp, Xq (Forozan *et al.* 2000). The authors also reviewed the existing CGH data from primary breast tumors (altogether 698 samples) and discovered that many of the genetic changes detected in breast cancer cell lines were similar to those found in primary breast cancers. Although several of these chromosomal regions have been designated to contain a putative tumor suppressor gene, the actual number and identity of these genes relevant to breast carcinogenesis is currently unknown.

2.6.2 Chromosome 11q loss of heterozygosity target candidate genes in breast cancer

Chromosome 11q is one of the most common targets for allelic loss in human cancers (Seizinger *et al.* 1991). In breast cancer, high LOH frequencies have been reported by several investigators (Hampton *et al.* 1994, Winqvist *et al.* 1995, Kerangueven *et al.* 1997a,b, Koreth *et al.* 1997, Negrini *et al.* 1995, Laake *et al.* 1997). Together with CGH studies, in which a loss of chromosome 11q in primary breast tumors has also been seen on a regular basis (Isola *et al.* 1995, Courjal & Theillet 1997, Kuukasjärvi *et al*. 1997, Schwendel *et al*. 1998, Tirkkonen *et al.* 1998, Roylance *et al.* 1999), these observations suggest the presence of one or more tumor suppressor genes within this region.

The presence of a crucial tumor suppressor gene is further supported by the observation that introduction of a normal chromosome 11 can reverse the tumorigenic and metastatic potential of MCF-7 and MDA-MB-435 breast cancer cell lines, respectively (Negrini *et al.* 1994, Phillips *et al*. 1996). In addition, transfer of the normal chromosome 11 suppresses tumorigenicity in Wilms' tumor, lung, rhabdomyosarcoma, cervical, and melanoma cell lines (Weissman *et al*. 1987, Koi *et al*. 1989, Oshimura *et al*. 1990, Satoh *et al*. 1993, Robertson *et al*. 1996). Certain chromosome 11q22-q23 fragments represented by YAC clones are also known to significantly restrain *in vivo* tumor formation (Murakami *et al*. 1998, Koreth *et al*. 1999).

In breast tumors, there are three commonly deleted regions in chromosome 11q. The most proximal region maps to 11q22.3-q23.1 (Hampton *et al.* 1994, Negrini *et al.* 1995, Kerangueven *et al.* 1997b, Koreth *et al.* 1997, Laake *et al.* 1997), and contains for instance the genes *ATM*, *DDX10*, *PPP2R1B* and *SDHD* (Savitsky *et al.* 1995a, Savitsky *et al*. 1996, Wang *et al*. 1998, Baysal *et al.* 2000). The second region, 11q23.2-q23.3 (Negrini *et al.* 1995, Kerangueven *et al.* 1997b, Launonen *et al*. 1999), harbors genes such as *DDX6*, *TSLC1* and *ALL1* (Lu & Yunis 1992, Baffa *et al.* 1995, Kuramochi *et al.* 2001). The most distal region is situated at 11q24 (Negrini *et al.* 1995, Kerangueven *et al.* 1997b). In addition to *CHK1*, this region contains a putative TSG called *ST14* (Cao *et al.* 1997, Zhang *et al.* 1998b) and also the genes *PIG8* and *P53AIP1*, which are both regulated by p53 (Polyak *et al*. 1997, Oda *et al*. 2000).

2.6.2.1 Genes related to DNA repair

Besides *ATM* (11q22.3) and *MRE11A* (11q21) (see sections 2.5.1.2 and 2.5.1.3), *CHK1* (11q24.2) is another candidate gene in chromosome 11q21-q24 directly related to cell cycle regulation and DNA repair. *CHK1* is a protein kinase, which acts to integrate signals from ATM and ATR (Flaggs *et al.* 1997, Sanchez *et al.* 1997) (see also 2.5). Of the two sensors, ATR is the main regulator of CHK1 in response to DNA damage, probably by phosphorylating Ser345 (Liu *et al.* 2000, Lopez-Girona *et al.* 2001). CHK1 phosphorylates and inhibits both CDC25A and CDC25C in response to DNA damage, thus assisting the arrest at several stages of the cell cycle (Sanchez *et al.* 1997, Falck *et al.*

2001b). Mice with a homozygous *Chk1* mutation die during embryogenesis, whereas mice carrying a heterozygous *Chk1* mutation are healthy (Liu *et al.* 2000). However, crossing with *WNT1* transgenic mice results in modestly enhanced tumorigenesis, which implies involvement of the ATR/CHK1 pathway in tumor suppression (Liu *et al.* 2000). Somatic mutations in both *ATR* and *CHK1* have been observed in gastric tumors with microsatellite instability (MSI) (Menoyo *et al.* 2001). *CHK1* mutations associated with a high degree of MSI were also found in colorectal and endometrial tumors (Bertoni *et al.* 1999).

2.6.2.2 Other candidate genes

PPP2R1B (11q23.1) (Wang *et al.* 1998) and *TSLC1* (11q23.2) (Kuramochi *et al.* 2001) are other candidate genes on 11q21-q24 that have been linked to tumorigenesis. The protein phosphatase 2A (PP2A) is one of the major cellular serine-threonine phosphatases (Wera & Hemmings 1995), and it is involved in pathways related to cellular metabolism, DNA replication, transcription, translation, cell-cycle progression, RNA splicing and several other processes (Janssens & Goris 2001, Wera & Hemmings 1995). It is a heterotrimeric holoenzyme generated by the association of a 36 kDa catalytic subunit and a 65 kDa structural subunit A with variable regulatory subunits. The regulatory subunit forms the scaffold of the holoenzyme and exists in two isoforms, α and β, which share 86% amino acid identity (Hemmings *et al.* 1990). These α and β isoforms are encoded by different genes, *PPP2R1A* and *PPP2R1B,* respectively.

In early G2, PP2A inhibits the complete CDC25C phosphorylation and activation, thus preventing the activation of CDC2-cyclin B1 complex and entry to mitosis (Janssens & Goris 2001). It is also required to keep the CDC2-cyclin B1 in inactive form by positively regulating the activity of the kinase necessary to phosphorylate CDC2 on Tyr15 (Kinoshita *et al*. 1993, Dunphy 1994). In addition, PP2A may have a role in the exit from mitosis by participating in cyclin B destruction and by activating specific mitotic substrates of the activated CDC2-cyclin B complex (Janssens & Goris 2001). Furthermore, PP2A might also function in apoptosis, as suggested by its interactions with caspase-3 and BCL2 (Deng *et al.* 1998, Santoro *et al.* 1998, Ruvolo *et al.* 1999). It has also been shown to inhibit nuclear telomerase activity in breast cancer cells (Li *et al.* 1997). Interestingly, cancer-associated mutations in both isoforms of the regulatory subunit, *PPP2R1A* and *PPP2R1B*, have been identified (Wang *et al.* 1998, Calin *et al.* 2000).

TSLC1 (also called *IGSF4*) is transcribed into a 1.6- or 4.4-kb mRNA and encodes a ubiquitously expressed transmembrane glycoprotein of 442 amino acids (Gomyo *et al.* 1999), which localizes to perinuclear and plasma membranes. TSLC1 contains three immunoglobulin-like C2-type domains, one transmembrane domain and a short cytoplasmic domain. The extracellular domain shows significant homology to those of immunoglobulin superfamily proteins, including NCAM1 and NCAM2, as well as to mouse IGSF-B12 (Gomyo *et al.* 1999). Also, the cytoplasmic domain is highly homologous with that of glycophorin C, a protein required for anchoring protein 4.1 in

the red blood cells (Marfatia *et al.* 1995). Thus, TSLC1 might play an important role in the formation of certain cell-to-cell or cell-to-substrate junctions (Kuramochi *et al.* 2001, Fukuhara *et al.* 2001). Also, based on their structural homology, two *TSLC1* family members, *TSLL1* and *TSLL2*, have recently been isolated (Fukuhara *et al.* 2001). Unlike *TSLC1*, however, these two genes are expressed in several specific tissues, most abundantly in the prostate, brain and kidney, and to a lesser extent in other tissues.

TSLC1 resides in presumably the most important 100-kb subsegment of the larger 700-kb genomic region shown to suppress completely the tumorigenicity of the human non-small-cell lung cancer (NSCLC) cell line A549 (Murakami *et al.* 1998). Truncation of the cytoplasmic domain of TSLC1 in a primary NSCLC tumor suggests that this domain is important for tumor suppression activity (Kuramochi *et al.* 2001). However, mutational inactivation of TSLC1 was found to be rare in tumors exhibiting LOH, but instead, frequent downregulation of TSLC1 expression through promoter region hypermethylation was observed (Kuramochi *et al.* 2001).

2.6.3 Alternative mechanisms of gene inactivation

In addition to qualitative alterations, which modify genes structurally, gene function can also be disrupted quantitatively through epigenetic alterations by changing the level of gene expression. For example, the altered intensity of DNA methylation on some important regulatory regions can affect the gene function. Following the change in methylation level, MeCP2 (methyl-CpG-binding protein 2) attaches to methylated DNA in a sequence independent fashion recruiting a complex, which contains a transcriptional co-repressor and a histone deacetylace (Nan *et al.* 1998, Jones *et al.* 1998). Subsequently, two possible mechanisms have been hypothesized for histone deacetylation to cause changes in chromatin structure. It could either allow ionic interactions between the positively charged amino-terminal histone tails and the negatively charged DNA backbone, which interferes with the binding of transcription factors to their specific recognition sequences, or lead to chromatine compaction through favoring interactions between adjacent nucleosomes (Bestor 1998). Eventually, these modifications lead to altered level of gene expression.

The methylation patterns of normal and cancerous cells differ from each other in many ways. Of the known mammalian DNA methyltransferases (DNMT), DNMT1 is generally responsible for maintenance of methylation, whereas *de novo* methylation is due to DNMT3a and DNMT3b. In malignant cells, the normal activities of these methyltransferases are dysregulated (Okano *et al.* 1999, Yokochi & Robertson 2002). Due to altered activity of the DNMT enzymes, both global hypomethylation and intense hypermethylation at normally unmethylated regions are observed in tumor cells (Baylin & Herman 2000, Esteller 2000). The amount of methylated CpGs decreases with age, and the rate of loss of these residues appears to be inversely related to the life span (Wilson *et al.* 1987). As the correct methylation pattern is essential for proper gene expression and the maintenance of genomic integrity, as a result of hypomethylation, the risk of accumulating cancer-related expression changes also increases. For example, in

demethylated somatic cells, the loss of DNMT1 changes the expression of several genes indicated in cell cycle control and signal transduction and also results in p53-dependent apoptosis (Jackson-Grusby *et al.* 2001). Indications of global hypomethylation have been reported in both hereditary and sporadic breast tumors (Esteller *et al.* 2001b).

Local hypermethylation is the most widely studied altered methylation pattern. Silencing of gene transcription is associated with gains of DNA methylation in normally unmethylated gene promoter regions (Jones & Laird 1999, Baylin & Herman 2000). In contrast to the genome in general, which is scarce of CpGs, approximately half of the gene promoters contain a stretch of DNA rich in CpG dinucleotides. These CpG islands are unmethylated in normal cells, apart from the imprinted genes and the inactive X chromosome in women. Their unmethylated state allows the expression of the adjacent genes in the presence of appropriate transcription factors. In cancerous cells, however, several of these CpG islands become hypermethylated, and as a consequence, shut down the expression of the corresponding gene.

In addition to CpG methylation, another site of methylation $C^{m}C$(A/T)GG, which has previously been reported only in retroviral gene silencing after viral integration into the mammalian genome (Lorincz *et al.* 2000), has now also been found to control promoter activity in mammalian cells (Malone *et al.* 2001). However, whether the $C^{m}C$(A/T)GG marks genetic elements for transcriptional silencing similarly to CpGs, or whether they protect CpG islands from CG methylation needs to be investigated in more detail (Lorincz & Groudine 2001).

To date, in various neoplasias several aberrantly methylated genes have been reported, including many of the genes associated with DNA repair: *MLH1*, *MGMT*, *BRCA1* and *GSTP1* (Lee *et al.* 1994, Dobrovic & Simpfendorfer 1997, Herman *et al.* 1998, Esteller *et al.* 1999, Esteller *et al.* 2000). Promoter hypermethylation seems to be a key feature of all major tumor types (Esteller *et al.* 2001a), and although many tumors share the same changes for some particular genes, unique profiles do exist for different tumor types. These profiles will provide important knowledge of the critical cancer-related cellular pathways and thus facilitate the development of molecular detection strategies for different cancer types.

3 Outlines of the present study

Despite active efforts, the existence of additional major high-penetrance cancer susceptibility genes with similar harmful effects in diverse geographical populations worldwide as has been observed for *BRCA1* and *BRCA2* remains questionable. As the defects in DNA repair and cell cycle control are known to promote carcinogenesis, a proportion of inherited breast cancers might be attributable to mutations in the genes involved in these mechanisms. Therefore, we wanted to assess the role of germline mutations in some of the central genes in the DNA damage response pathway, which are also associated with familial cancer predisposing syndromes. Furthermore, the tracking of genes important for tumorigenesis in sporadic disease might also open up new perspectives into familial breast cancer. Against this background, the specific aims of this study were to:

1. Investigate the contribution of germline mutations in the two known genes associated with the Li-Fraumeni syndrome, *TP53* and *CHK2*, to breast cancer predisposition in Finland.

2. Examine the prevalence of the *ATM* germline mutations previously detected in Finnish ataxia-telangiectasia families among breast cancer patients.

3. Evaluate the role of putative target genes of allelic loss in chromosome 11q21-q24, and along with intragenic mutations, also to investigate CpG island hypermethylation as a mechanism of gene expression silencing.

4 Materials and methods

4.1 Patients

4.1.1 Studies I-II

One hundred and eight Finnish *BRCA1* and *BRCA2* mutation-negative breast cancer families were analyzed for *TP53* mutations. Geographically, 79 families came from the Oulu University Hospital District, 13 from the Tampere University Hospital District and 16 from the Helsinki University Hospital District. For *CHK2* mutations analysis, only the families from the Oulu University Hospital District were selected. The inclusion criteria were one or more of the following: 1) at least three (two in combination with other selection criteria) cases of breast cancer in first- or second-degree relatives, 2) early disease onset (≤35 years alone, or <45 in combination with other inclusion criteria), 3) bilateral breast cancer, or 4) multiple tumours including breast cancer in the same individual. A proportion of the studied families also fulfilled the criteria for Li-Fraumeni or Li-Fraumeni-like syndromes (Birch *et al.* 1994, Eng *et al.* 1997) (Table 3).

4.1.2 Study III

A total of 215 breast cancer patients from 162 families from Central and Northern Finland were chosen for *ATM* mutation screening. All families had indications of moderate to high genetic susceptibility to breast cancer, basically fulfilling the same selection criteria than in studies I and II. Most of these patients had previously been excluded for *BRCA1*, *BRCA2* and *TP53* mutations by using extensive PCR-based screening methods (Huusko *et al*. 1998, Huusko *et al.* 1999, study I). Additionally, 85 sporadic breast cancer cases from the Oulu area were included in the study. Reference

blood samples from 200 geographically matched controls were used to validate the observations from the two test groups.

Table 3. Summary of the breast cancer families' phenotypes (I,II)

Phenotype	Number of families
TP53 study	
All studied families	108
Families with implications of hereditary breast cancer	75
Breast cancer/LFL families	32
Breast cancer/LFS families	1
CHK2 study	
All studied families	79
Families with implications of hereditary breast cancer	58
Breast cancer/LFL families	20
Breast cancer/LFS families	1

4.1.3 Study IV

Breast tumor DNAs from 31 sporadic cases were selected to elucidate the role of suitable candidate genes in chromosome 11q21-q24. All patients showed loss of heterozygosity (LOH) in chromosomal region 11q23 (Laake *et al.* 1999, Launonen *et al.* 1999). In three patients, LOH had been detected only distally from the *ATM* locus on 11q (marker *APOC3*), but in the remaining 28 cases LOH occurred both in the *ATM* region and at least with one additional microsatellite marker (*D11S1819, D11S1778, D11S2179, D11S1294, D11S1818, D11S927*).

4.2 DNA extraction

Extractions of DNA from blood lymphocytes and tissue material derived from breast cancer patients as well as from control blood samples (I, II, III, IV) were performed using standard phenol-chloroform methods.

4.3 Mutation analysis

4.3.1 Conformation-sensitive gel electrophoresis (CSGE) (I-III)

Conformation-sensitive gel electrophoresis (CSGE) was the key method utilized in mutation screening to identify germline alterations in the studied genes. CSGE analysis is based on the assumption that even a single base mismatch can produce a conformational change in the DNA double helix (Ganguly *et al.* 1993). When a PCR product containing a mismatch is first denatured and then allowed to reanneal, the randomly forming homo- and heteroduplexes can be distinguished by their differential migration on a mildly denaturing polyacrylamide gel.

Each exon and the corresponding splice sites were PCR-amplified under specifically optimized reaction conditions. In general, reactions were carried out in a total volume of 10 µl, containing 50 ng of genomic DNA as a template, 1 x PCR buffer (1.5-2.5 mM $MgCl_2$), dNTPs (200 µM each), 20 pmol of each primer and 0.5 U of AmpliTaqGold polymerase (Applied Biosystems). The conditions for PCR were: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 50-65 °C for 1 min, extension at 72 °C for 1 min 30 s; and final extension at 72 °C for 10 min. Following the amplification, the samples were subsequently denaturated at 98 °C for 5 min, and kept at 68 °C for 30 min to allow DNA heteroduplex formation. The samples were then mixed with 6 µl of 10 x loading buffer (containing 30% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanol) and loaded onto a 10% CSGE gel (containing 25% polyacrylamide, 99:1 ratio of acrylamide to 1,4-bis(acryloyl)piperazine, 10% ethylene glycol, 15% formamide and 0.5% Tris-Taurine-EDTA-buffer) and electrophoresed at 400 V for 16-22 h, depending on the length of the analyzed fragment. After electrophoresis, the gel was stained on the glass plate in ethidium bromide, visualized in UV light and photographed.

4.3.2 DNA sequencing (I-IV)

Samples with putative alterations detected in CSGE were re-amplified with the same set of oligonucleotides, but in a larger reaction volume of 30 µl. The PCR product was purified with the QIAquick PCR purification Kit (Qiagen). For sequencing reactions, the SequiTherm EXCEL™II DNA Sequencing Kit-LC (Epicentre Technologies) was utilized. Basically, a mix of 100-200 fmoles of purified PCR product, sequencing buffer, IRD-labeled primers (2 pmoles each) and 5 U of EXCEL II Polymerase was prepared to a total volume of 17 µl. Into four tubes containing 2 µl of each termination mix, 4 µl of reaction mix was added. The reaction conditions were: intial denaturation at 92 °C for 2 min; 30 cycles of denaturation at 92 °C for 30 s, annealing at 50-65 °C for 30 s, extension at 72 °C for 30 s. The samples were then mixed with 3 µl of loading buffer included in the SequiTherm EXCEL™II DNA Sequencing Kit-LC (Epicentre Technologies) and

denatured at 96 °C for 3 min prior to electrophoresis, which was performed with the Li-Cor IR^2 4200-S DNA Analysis system (Li-Cor Inc.). Of each sample, an aliquot of 0.5-1 µl was loaded on to a 6% Long Ranger gel and run at 1500 V for 7-9 hours, depending of the length of the analyzed fragment. The data were analyzed using the AlignIR™ and BaseImagIR™ softwares (Li-Cor Inc.).

4.4 Methylation analysis (IV)

In sodium bisulfite sequencing technique, the unmethylated cytosines are first converted to uracils by sodium bisulfite under conditions in which methylated cytosines remain nonreactive (Frommer *et al.* 1992). When the modified DNA is subsequently PCR-amplified and sequenced, all the uracil and thymine residues amplify as thymine. On the contrary, the methylated cytosine residues amplify as cytosine, and can be therefore discriminated from the unmethylated cytosines.

Sodium bisulfite treatment was performed as described by Clark *et al.* (1994). First, 2 µg of tumor DNA was denatured with 0.3 M NaOH (37 °C, 15 min), reacted with freshly prepared 3.6 M sodium bisulfite and 1 mM hydroquinone (55 °C for 14 h) and desalted by using a Wizard DNA Clean Up kit (Promega) according to manufacturer's instructions. Subsequently, 3 N NaOH was added to a final concentration of 0.3 N to desulphonate DNA. Finally, bisulfite-treated DNA was ethanol-precipitated, dried and eluted with TE buffer prior to PCR.

The gene regions of interest were PCR-amplified using bisulfite-treated DNA as a template. The products were ran on 1% agarose gel, excised and purified with a QIAquick Gel Extraction Kit (Qiagen), and cloned into the pGEM-T-easy vector (Promega). The vectors were transformed into chemically competent *E.coli* TOP10F' cells (Invitrogen) following a standard transformation protocol. The transformed cells were grown overnight on agar plates containing ampicillin, IPTG and X-gal. Based on the blue/white selection, at least ten positive recombinants were inoculated into 3 ml of LB broth containing ampicillin and grown overnight with vigorous shaking. Bacteria were harvested and plasmids extracted by using the Wizard®PlusMiniprep Kit (Promega).

DNA sequencing of the clones was performed in essentially a similar way as described above, except that M13 primers were used to amplify the cloned fragments. Also, in addition to utilizing commercial software for data analysis, the methylation status of each analyzed fragment was evaluated by at least two investigators.

4.5 Statistical analysis (II, IV)

The observed differences in mutation frequencies between the studied samples from breast cancer patients and the control samples were analyzed in a Bayesian framework (II) (Gelman *et al*. 1995). Unlike the Chi-square test, the Bayesian approach provides the probabilities for the presented hypothesis to be both true and false. Furthermore, in the

Bayesian model, none of the expected values are fixed, which results in a more plausible statistical estimate. The probability model was set up by assuming that the number of mutations follows the Poisson distribution with a mean $\lambda_i=\theta_i N_i$, given the number of individuals is N_i and the mutation frequency is θ_i. Also, θ_i was assumed to follow Beta (1,1)=Unif (0,1) distribution. Formally:

$$x_i \mid N_i,\theta_i \sim \text{Poisson}(\theta_i N_i)$$
$$\theta_i \sim \text{Beta}(1,1)$$

The comparisons of mutation frequencies between the different groups were performed by calculating the ratio of the frequencies, $R_{i,j}=\theta_i/\theta_j$. The posterior distributions of the model parameters were obtained by Monte Carlo Markov Chain simulation, which was carried out with the WinBUGS 1.3 software. Furthermore, for H_0 (estimating how well the frequency observed in one group equals that in the reference group), traditional Chi-square test calculations were performed using p=0.01 as cut-off value for statistical significance.

The Chi-square test for linear trend and Fisher's two-tailed exact test were used for the statistical evaluation of association between methylation and clinical variables (IV).

5 Results

5.1 Analysis of germline mutations

The mutation analysis of *TP53*, *CHK2* and *ATM*, which are all central genes in DNA DSB response pathway, was performed to investigate the possibility that germline mutations in these genes could explain at least a fraction of inherited breast cancer susceptibility in families lacking mutations in *BRCA1* and *BRCA2* genes.

5.1.1 TP53 (I)

Among the 108 breast cancer families studied, one new family with a *TP53* mutation, Arg248Gln, was identified. In addition, a silent variant (Arg213Arg) and several intronic polymorphisms were detected. The family carrying Arg248Gln showed a strong family history of breast cancer (Table 4), but also matched the criteria for LFL. Together with the previously identified two families (Huusko *et al.* 1999), 2.8% (3/108) of the studied breast cancer families were found to carry a *TP53* mutation, all of them also meeting the criteria for LFS or LFL.

5.1.2 CHK2 (II)

When screening for *CHK2* mutations, the only alteration of interest seen in the exonic regions of the gene was Ile157Thr. It was observed in 8.9% (7/79) of the studied breast cancer families (Table 4). Four of these seven families met the criteria for LFL. However, in two of the mutation-positive families, where suitable multiple DNA specimens were available for analysis, the mutation segregated ambiguously with the cancer phenotype.

Furthermore, Ile157Thr was found in 6.5% (13/200) of the anonymous cancer-free blood donors and 3.9% (10/259) of the unselected breast cancer cases.

Table 4. Germline sequence alterations in the p53, CHK2 and ATM genes in Finnish families with breast cancer (I-III)

Gene	Mutation	aa change	Status[a]	Family ID	Phenotype[b,c]
TP53	659A>G	Tyr220Cys[d]	M	020	Bil Br(36) + Os(13), Bt, Cpp(4), Lu, Pan, Os(19)
	703A>G	Asn235Ser[d]	M	018	Bil Br(57), Br(42), Br, Br, Csu, Ep, Sto, Sto, Sto
	743G>A	Arg248Gln	M	6001	Br(22), Bil Br (32) + Li(29), Sa(54), Bt
CHK2	470T>C	Ile157Thr	U	1	Br(51), Br, Br
	470T>C	Ile157Thr	"	2	Br(64), Br(65), Ov
	470T>C	Ile157Thr	"	3	Bil Br(40), Br(80), Br(46), Br(54), Sk(42), Csu
	470T>C	Ile157Thr	"	4	Br(36), Br(54), Csu
	470T>C	Ile157Thr	"	5	Br(47), Br(50), Br(80), Bo(89), Sto
	470T>C	Ile157Thr	"	6	Br(50), Br(54), Br(50), Bo(70), Leu(41), Pro
	470T>C	Ile157Thr	"	7	Br(40), Br(49), Br(64), Br(52), Br(72)
ATM	133C>T	Arg45Trp	P	001	Bil Br(45)
	146C>G	Ser49Cys	P	002	Br(48), Br(58), Br(60), Pan
	7522G>C	Ala2524Pro	M	003	Br(50), Br(59), Br(51), Th(47), Pro(59), Br (71), Sto, Lu (55), Csu
	7522G>C	Ala2524Pro	M	005	Br(57) + Sto(41), Bs(70), Sto, Sto, Sto(41), Csu, Th(55)
	6903insA	loss of 685 3'terminal aa	M	004	Br(50), Br(47) + Sar(18), Br(40), Sto, Bt, Pan(40), Br(54), Sto, Pan, Br(45)[e], Br(37)[e], Bt, Sto, Sto, Br(30), Bt, Pro, Sto

[a]M, disease associated mutation; U, disease-association currently unconfirmed; P, polymorphism.
[b]Bil Br, bilateral breast; Br, breast; Bo, bone; Bt, brain; Cpp, choroids plexus papilloma; Csu, cancer site unknown; Leu, leukemia; Li, liver; Lu, lung; Os, osteosarcoma; Ov, ovary; Pan, pancreas; Pro, prostata; Sa, sarcoma; Sk, skin; Sto, stomach; Th, thyroid; Ep, ependymoma.
[c]Age at diagnosis is given in brackets when known. The + sign indicates more than one primary tumor in the same individual.
[d]Reported in Huusko *et al.* (1999)
[e]*BRCA2* mutation carrier

The hypothesis that the mutation frequency among hereditary breast cancer patients would be different from the mutation frequency in a reference group was tested using the Bayesian model. The minimum value to prove that the observed incidence was higher than expected was 0.99, and none of the calculated probabilities for mutation frequencies reached this value. The obtained values were 0.78 (breast cancer families vs. cancer-free

blood donors), 0.11 (cancer-free blood donors vs. unselected breast cancer cases), and 0.96 (breast cancer families vs. unselected breast cancer cases). To estimate how well the frequency observed in one group equals that in a reference group, traditional Chi-square test calculations were made. The obtained values were 0.72 (p=0.395), 2.96 (p=0.085) and 5.53 (p=0.019), respectively, being thus statistically insignificant.

Due to the presence of homologous copies of the 3'part of the *CHK2* gene (Sodha *et al.* 2000), atypical banding in CSGE was observed for the exons 10-14. As the CSGE analysis is based on homo- and heteroduplex formation between wild-type and mutated alleles, based on the appearance of new heteroduplex bands, it is possible to detect more than one kind of mismatch in a given PCR product (Körkkö *et al.* 1998). Therefore, instead of a single band (e.g. homoduplex) indicating a lack of mutation, genomic loci co-amplified in PCR with the exons 10-14 in CSGE analysis resulted in additional bands (e.g. one or more heteroduplexes). For that reason, the screening for samples displaying a banding pattern different from the others in CSGE provided some leads as to whether the analyzed exons contained sequence alterations or not. The banding patterns for the exons 10-14, however, were similar in all the screened DNA samples (data not shown).

5.1.3 ATM (III)

Two of the eight *ATM* germline mutations previously identified in Finnish AT families were found among the studied cohort of breast cancer patients with a hereditary disease background (Table 4). 7522G>C was identified in two women with breast cancer belonging to separate cancer families (003 and 005). The other alteration, insertion of adenine (6903insA), was found in three sisters who all had breast cancer. Neither 6903insA nor 7522G>C was seen among the studied sporadic breast cancer cases or the control samples, indicating that these two *ATM* mutations in addition to being instrumental to AT, could also be related to a hereditary predisposition to breast cancer. Altogether, putative disease-associated *ATM* mutations were detected in 1.9% (3/162) of the studied families displaying breast cancer.

Other sequence variants, two in exon 5 (133C>T and 146C>G) and one in the intronic region between the exons 62 and 63, were all regarded as polymorphisms. 133C>T leads to an amino acid substitution Arg45Trp, and it was observed in a woman with bilateral breast cancer at age 45. The second exon 5 variant, 146C>G, results in Ser49Cys and was detected in a woman diagnosed with breast cancer at age 60. She had two sisters with breast cancer at ages 48 and 58, but neither of them carried the alteration. We did not observe either of these alterations in the sporadic breast cancer group or the healthy controls. Neither Arg45Trp nor Ser49Cys reside in a known functional ATM domain, and are thus less likely to interfere with ATM kinase function. The third sequence alteration, IVS62+8A>C, was detected in 2.3% (5/215) of the familial breast cancer patients, 2.4% (2/85) of the sporadic breast cancer cases and 2.0% (4/200) of the controls.

5.2 Analysis of somatic aberrations in chromosome 11q21-q24 (IV)

Based on the previous LOH findings (Laake *et al.* 1999, Launonen *et al.* 1999), the role of putative target genes of allelic loss in chromosome 11q21-q24 was evaluated. The location of the markers used previously for the LOH analysis (*D11S1819, D11S1778, D11S2179, D11S1294, D11S1818, D11S927, APOC3*) and the studied LOH candidate target genes are shown in Figure 4. For *MRE11A*, *CHK1*, *PPP2R1B* and *TSLC1* genes, both the presence of intragenic mutations and the level of CpG island hypermethylation were determined. In addition, the *ATM* gene was included to the methylation analysis.

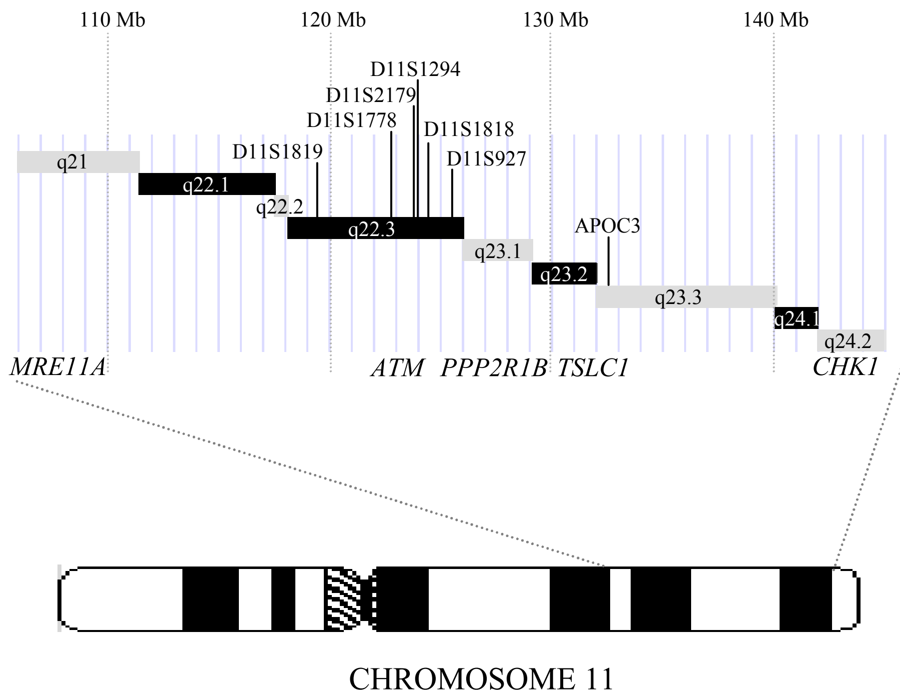

Fig. 4. Locations of the studied genes and the utilized microsatellite markers in chromosome 11q21-q24. Adapted from http://www.ncbi.nlm.nih.gov/genome/guide/HsChr11.shtml and http://genome.ucsc.edu.

5.2.1 Mutation analysis

In the four analyzed genes, *MRE11A*, *CHK1*, *PPP2R1B* and *TSLC1*, only one putative cancer-associated mutation was found. However, this missense mutation, Lys354Arg in *PPP2R1B* exon 9, was also present in the corresponding normal tissue sample, but not in any of the 200 controls. Both lysine and arginine are hydrophilic basic amino acids, and very similar in structure. Therefore, despite the conserved position of this residue in the HEAT (huntingtin-elongation-A subunit-TOR) repeat 9, the presence of either of the two amino acids appears to result in an equally functional protein (Groves *et al.* 1999).

5.2.2 Methylation analysis

All analyzed CpGs of *MRE11A*, *CHK1*, *PPP2R1B*, and *ATM* were found to be unmethylated. For *TSLC1*, however, a high proportion of the tumors (33%, 10/30) displayed multiple methylated cytosines in at least one of the analyzed clones. Especially three of these tumors showed considerably elevated levels of methylation at the six studied CpG sites (Figure 5). The number of clones with methylated cytosines inversely correlated with the percentage of LOH. In normal breast tissue and blood lymphocytes, these CpGs were unmethylated. *TSLC1* methylation was not related to any clinicopathological characteristics of the tumors.

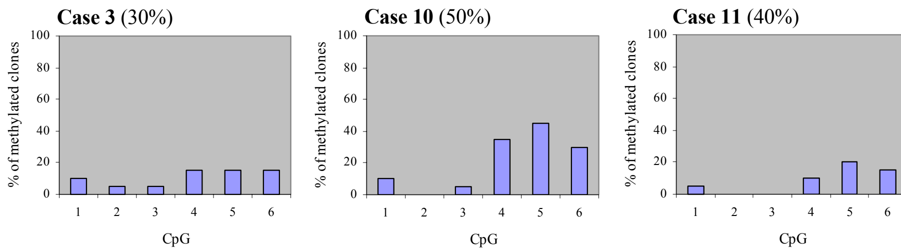

Fig. 5. *TSLC1* promoter methylation status in tumors from three breast cancer patients. The corresponding LOH percentage is shown next to the case number.

6 Discussion

6.1 Germline mutations in Finnish breast cancer patients

6.1.1 TP53 and CHK2 (I, II)

Earlier studies on Finnish hereditary breast cancer patients (Vehmanen *et al.* 1997a,b, Huusko *et al.* 1998) have implied a smaller contribution of *BRCA1* and *BRCA2* mutations in Finland compared with other Western European populations. Therefore, one possible way to track additional inherited genetic changes resulting in a predisposition to breast cancer was to investigate whether germline mutations in the LFS and LFL-associated genes, *TP53* and *CHK2*, could be found in breast cancer families. Breast cancer is the most prominent type of cancer in these related cancer syndromes (Kleihues *et al*. 1997), implying that germline mutations in the same genes might also predispose to the early onset breast cancer, even in families lacking the other typical cancer signatures of LFS.

In our initial study, the exons 5-8 of the *TP53* gene were screened in seven families, two of them exhibiting a LFS and five a LFL phenotype (Huusko *et al.* 1999). Two *TP53* missense mutations were detected, Tyr220Cys in an LFS family, and Asn235Ser in an LFL family. Subsequently, we screened a cohort of 108 Finnish breast cancer families for *TP53* mutations. In this more detailed follow-up study, we included the entire coding region (exons 2-11) and also the flanking intronic regions. As a result, one additional *TP53* mutation, Arg248Gln, was detected in a breast cancer family that also exhibited the typical features of LFL. Arg248Gln appears to be the most frequently found mutation in LFS (Shibata *et al.* 1996)

Two diverse classes of mutations have been distinguished based on *in vitro* assays and the three-dimensional structure of the p53 protein (Cho *et al.* 1994). Class I mutations alter the amino acids directly involved in interactions between DNA and proteins. They maintain the wild-type conformation, but do not bind the HSP70 heat-shock protein (Hinds *et al.* 1990, Ory *et al.* 1994). On the contrary, the conformations resulting from class II mutations have been altered due to the genetic change, and they bind HSP70

intensively. Arg248Gln belongs to class I, and structurally it resides in a highly conserved region, L3, which is essential for p53 DNA-binding functions (Cho *et al.* 1994). In clinical studies, breast cancer patients with somatic mutations in the L2/L3 domain displayed shorter survival (Børresen *et al.* 1995, Berns *et al.* 1998, Gentile *et al.* 1999) as well as a poorer response to tamoxifen (Berns *et al.* 2000) and doxorubicin (Aas *et al.* 1996, Geisler *et al.* 2001) than patients with other types of mutations or wild-type *TP53*.

The proportion of *TP53* mutations (2.6%, 3/108) derived from our two studies is consistent with the earlier data (Børresen *et al*. 1992, Sidransky *et al*. 1992). Furthermore, *TP53* mutations occur mainly at specific mutation-prone regions of the conserved parts of the gene. In a small proportion of breast cancer patients, the increased risk of inherited breast cancer can be explained by heterozygous germline *TP53* mutations. Apart from breast cancer, these mutation-positive families usually also exhibit other cancer types characteristic of LFS and LFL.

Recently, *CHK2* germline mutations were identified in LFS families lacking mutations in *TP53*. As Bell *et al.* (1999) originally screened four LFS and eighteen LFL cases and found three distinct *CHK2* alterations (1100delC, Ile157Thr and 1422delT) in three of the studied families (13.6%, 3/22), a similar incidence of *CHK2* mutations was initially expected among the 21 LFL and LFS families included in our study. However, the first of the mutations detected by Bell *et al.* (1999), 1422delT, was subsequently shown to be an artifact due to homologous duplications of the 3'end of the genomic sequence (exons 10-14) (Sodha *et al.* 2000). The second mutation, 1100delC, occurred in a classical LFS family, but as the population prevalence of this alteration was demonstrated to be about 1%, it is not likely to be a high-penetrance allele for LFS susceptibility (The *CHEK2*-Breast Cancer Consortium 2002). In the same study, however, 1100delC was shown to result in a twofold increase of breast cancer risk in women and tenfold increase of risk in men (The *CHEK2*-Breast Cancer Consortium 2002). The third mutation, Ile157Thr, was seen in a single individual with three primary tumors (breast, melanoma and lung), but without a reported family history of cancer. This phenotype is therefore only suggestive of LFS or LFL (Birch *et al*. 1994, Eng *et al*. 1997, Vahteristo *et al.* 2001b).

Among Finnish breast cancer families exhibiting a LFS or LFL phenotype, the only detected alteration within the protein-encoding region of the gene was Ile157Thr. Seven breast cancer families (8.9%, 7/79) were found to carry this missense mutation, and four of these families also met the criteria for LFL. However, the alteration was also present in the studied controls (6.5%, 13/200) as well as in unselected breast cancer cases (3.9%, 10/259). To conclusively exclude the presence of additional mutations in the exons 10-14, our negative result should be confirmed by long-range PCR based screening method developed for these exons (Sodha *et al.* 2002).

In Ile157Thr, the altered isoleucine is located within the forkhead-associated (FHA) domain, which is a highly conserved 60-amino acid protein interaction domain essential for the activation of the CHK2 yeast homolog Rad53 in response to DNA damage (Sun *et al.* 1998). The CHK2 protein carrying the Ile157Thr change has similar kinase activity, expression levels and subcellular localization as endogenous CHK2 (Wu *et al.* 2001). In addition, similarly to wild-type CHK2, the mutant protein is activated following gamma radiation. However, it was later shown that CHK2 forms a protein-protein complex with p53, whose abundance at the S-phase checkpoint increases upon IR exposure. This interaction requires an intact FHA domain of CHK2 as well as successful tetramerization

of p53 (Falck *et al.* 2001a, Lee & Chung 2001). Despite the fact that the CHK2 carrying Ile157Thr was able to undergo an activatory mobility shift upon IR, it was not able to bind p53 *in vivo*, and its *in vitro* kinase activity remained low after IR. Also, the defective CHK2 protein was unable to bind and phosphorylate CDC25A (Falck *et al.* 2001b).

It has been suggested that parallel mutations in cell cycle checkpoint genes *TP53* and *CHK2* might provide some additional selective advantage to the affected tumor cells (Falck *et al.* 2001a). A colon cancer cell line, HCT-15, concomitantly lacks functions of both CHK2 and p53 (Falck *et al.* 2001a). Additionally, a simultaneous mutation in *CHK2* and *TP53* has been reported in a patient with small-cell lung cancer (Haruki *et al.* 2000). Furthermore, cancer-associated *CHK2* mutations, 1100delC and Arg145Trp, showed loss of the wild-type allele in primary tumors, whereas Ile157Thr and Arg3Trp did not (Lee *et al.* 2001). On the contrary, a breast tumor harboring Ile157Thr demonstrated mutational inactivation of both *TP53* alleles, which was not the situation for the 1100delC and Arg145Trp cases (Lee *et al.* 2001). *CHK2* might also have similar effects with other mutated genes, as Vahteristo *et al.* (2001b) reported a *CHK2* mutation carrier harboring a germline mutation in the *MSH6* gene.

In addition to LFS and breast cancer, the contribution of germline and somatic *CHK2* defects has been studied in a variety of cancers (Bell *et al.* 1999, Bougeard *et al.* 2001, Lee *et al.* 2001, Vahteristo *et al.* 2001b). Yet, only a few mutations have been detected. The absence of germline mutations has earlier been reported in gastric cancer (Kimura *et al.* 2000). Infrequent somatic *CHK2* mutations have been found in acute myeloid leukemia, non-Hodgkin's lymphoma, small-cell lung cancer and osteosarcoma, all of which are cancer types associated with the LFS phenotype (Haruki *et al.* 2000, Hofmann *et al.* 2001, Tavor *et al.* 2001, Miller *et al.* 2002). On the other hand, no somatic mutations were found in malignant gliomas (Ino *et al.* 2000).

Altogether, we identified Ile157Thr in seven Finnish breast cancer families. Due to a high frequency in the studied controls as well as in unselected breast cancer cases and the ambiguous segregation of the mutation in two of the mutation-positive families, our results suggest that Ile157Thr would not, by itself, be a mutation resulting in predisposition to cancer. The functional evidence, however, suggests that Ile157Thr disturbs the protein function by interfering with the FHA domain binding ability. Therefore, it seems likely that the altered CHK2 might promote cancer formation, possibly in combination with mutations in other relevant genes consistent with the polygenic susceptibility model recently presented by Pharoah *et al.* (2002). Consequently, Ile157Thr variant might be a low-penetrance allele conferring susceptibility to breast cancer similarly to 1100delC (The *CHEK2*-Breast Cancer Consortium 2002). Compared to other studied populations, Ile157Thr seems to be enriched in the Finnish population (Bell *et al.* 1999, Lee *et al.* 2001, Vahteristo *et al.* 2001b). Thus, the clinical significance of Ile157Thr requires further investigations among Finnish cancer patients. Additional genes besides *TP53* and *CHK2* are likely to account for the cancer susceptibility observed in the remaining LFS and LFL families.

6.1.2 ATM (III)

The increased risk of breast cancer in *ATM* carriers has been demonstrated in many studies (Swift *et al.* 1987, Pippard *et al.* 1988, Athma *et al.* 1996, Stancovic *et al.* 1998, Inskip *et al.* 1999, Janin *et al.* 1999, Olsen *et al.* 2001). However, contradictory data also exist (Vorechovsky *et al.* 1996, FiztGerald *et al.* 1997, Bay *et al.* 1998, Chen *et al.* 1998), and the true nature of these observations has thus remained elusive. As recurrent *ATM* mutations have been reported in several countries and within many different ethnic groups (Gilad *et al.* 1996, Ejima & Sasaki 1998, Laake *et al.* 1998, Sasaki *et al.* 1998, Stancovic *et al.* 1998, Telatar *et al.* 1998), we anticipated that the previously identified ‘Finnish’ *ATM* germline mutations would also be the most likely mutations prevalent in Finnish breast cancer patients, provided that there is an association between *ATM* germline mutations and susceptibility to breast cancer. In three studied families with breast cancer (1.9%, 3/162), we detected two germline alterations potentially relating to breast cancer susceptibility, Ala2524Pro and 6903insA. Both of these detected mutations had previously been found in AT families, segregating with the disease, and were thus known for their pathogenic nature (Laake *et al.* 2000a). Also, the Finnish AT families displaying the Ala2524Pro or 6903insA alteration showed excessive cases of breast and other cancers.

Earlier, Stancovic *et al.* (1998) described two AT families in which a heterozygous missense mutation, Val2424Gly (7271T<G), was found to associate with an increased risk of breast cancer. Interestingly, the AT patients carrying the homozygous mutation exhibited a milder phenotype compared to the typical AT. Along with Val2424Gly, another *ATM* mutation, IVS10-6T<G, was suggested to associate with an increased cancer risk in patients with early-onset breast cancer who had been exposed to low-dose ionizing radiation and had survived for more than five years after being diagnosed with breast cancer (Broeks *et al.* 2000, Dörk *et al.* 2001). Subsequently, the relation of these two dominant-negative mutations with respect to the increased breast cancer risk was confirmed in a large population-based case-control study (Chenevix-Trench *et al.* 2002). The penetrance estimate of these mutations corresponded to an overall 15.7-fold risk for the development of breast cancer averaged up to age 70. As most studies have concentrated on sporadic breast cancer cases instead of cancer families and utilized screening methods preferentially detecting protein-truncating mutations (Ángele & Hall 2000), there might be more *ATM* germline mutations with similar effects yet to be discovered. Interestingly, less common *ATM* missense substitutions seem to be enriched among breast cancer patients (Dörk *et al.* 2001, Teraoka *et al.* 2001), supporting the hypothesis of two diverse populations of *ATM* heterozygotes (Khanna 2000, Meyn 1999), of which the combination of a wild-type allele and an allele containing a missense mutation would be more prone to cause cancer predisposition.

In addition, other lines of evidence support the role of *ATM* as a tumor suppressor gene. First, germline *ATM* mutations have been discovered in breast cancer patients carrying a parallel mutation in *BRCA1* or *BRCA2* (Teraoka *et al.* 2001). The frequency of *ATM* mutations in this group was somewhat elevated compared to the control group, but did not differ significantly when compared to the overall case group, which involved both *BRCA1* and *BRCA2* mutation carriers and non-carriers. Second, an *ATM* variant,

Asp1853Asn, was found to modulate the penetrance of *MLH1* and *MSH2* germline mutations (Maillet *et al.* 2000). The *MLH1* or *MSH2* mutation carriers, who concurrently carried the Asp1853Asn variant, had an eightfold risk of developing colorectal and other HNPCC-related cancers (Maillet *et al.* 2000). Along with this mutation, the Arg1054Pro substitution has been proposed to be a genetic modifier of breast cancer penetrance (Larson *et al.* 1998). Finally, as women developing early-onset bilateral breast cancer are likely to harbor germline mutations in certain susceptibility genes (Tsuda & Hirohashi 1995, Kollias *et al.* 2000), loss of the same allele at a specific chromosomal site could suggest a common origin of the two malignancies observed in one individual. Kollias *et al.* (2000) demonstrated concordant LOH at *D11S1778*, which maps to the *ATM* locus, in 25% of the informative bilateral breast cancer cases, which further supports the role of *ATM* as a TSG in this malignancy.

In conclusion, heterozygous *ATM* germline mutations seem to contribute in some extent to breast cancer predisposition in Finland. Interestingly, the cancer families carrying *ATM* germline mutations originated from the same geographical region as the AT families displaying corresponding mutations. Although this implies the possibility of a founder effect concerning the distribution of Ala2524Pro and 6903insA, due to the circular nature of the argument followed by the inclusion of only the mutations previously identified in Finnish AT families, a more extensive study will be needed to validate this preliminary lead.

6.2 Somatic alterations in chromosome 11q (IV)

The presence of one or more tumor suppressor genes in the long arm of chromosome 11 has been established on several occasions. Apart from the available LOH and CGH data, there are many functional transfection studies that further verify these observations (Negrini *et al.* 1994, Koreth *et al.* 1999). However, despite the efforts to hunt down the target genes contributing to malignant transformation and growth of tumor cells, these genes still remain to be identified. Therefore, we assessed the possible role of some of the most interesting candidate genes as putative targets in chromosome 11q21-q24 by analyzing tumor DNAs for gene inactivation through both genetic and epigenetic alterations.

First, for the *MRE11A* and *CHK1* genes, which are both involved in DNA DSB signaling, a comprehensive mutation analysis was performed. In the absence of somatic mutations, the two other candidate genes, *PPP2R1B* and *TSLC1,* were subsequently included in the study. The lack of somatic mutations in the coding region and splice junctions of all these genes supports the prevailing view of only a low frequency of intragenic cancer-associated alterations. A small number of mutations in *MRE11A* and *PPP2R1B* have been previously reported (Wang *et al.* 1998, Calin *et al.* 2000, Takagi *et al.* 2000, Fukuda *et al.* 2001). *CHK1* mutations seem to be absent in most of the studied tumor types (Bell *et al.* 1999, Semba *et al.* 2000, Vahteristo *et al.* 2001b), with the exception of two studies in which frameshift *CHK1* mutations in MSI-positive gastric, colon and endometrial tumors were observed (Bertoni *et al.* 1999, Menoyo *et al.* 2001).

Furthermore, only two inactivating *TSLC1* mutations were detected among 161 primary tumors and cell lines (Kuramochi *et al.* 2001).

Because all previous studies on *MRE11A*, *CHK1*, and *PPP2R1B* inactivation or dysfunction have concentrated on finding mutations within the protein-encoding region, the possible influence of promoter region CpG methylation of these putative TSGs in 11q21-q24 was subsequently evaluated. As it was known from previous studies (Rodriguez *et al.* 2002) that tumors presenting LOH at the *ATM* locus do not show significant involvement of mutations in the corresponding wild-type allele, the *ATM* gene was also included in the methylation study that was performed. Of the selected candidate genes, the studied regions of *MRE11A*, *CHK1*, *PPP2R1B*, and *ATM* were all found to be unmethylated. However, three tumors showed relatively high methylation levels at the analyzed CpG sites of the *TSLC1* gene. In these cases, methylation occurred in up to 45% of the analyzed clones. This percentage is likely to reflect the presence of contaminating stromal and other non-malignant cells in the sample, as well as heterogeneity within the tumor. Thus, the percentage of methylation in a pure tumor specimen might be considerably higher. The assumption was supported by the results of the LOH analyses, which showed traces of contaminating cells in all the three tumors (Laake *et al.* 1999, Launonen *et al.* 1999). Additionally, the average percentage of methylation inversely correlated with the percentage of contamination of non-malignant cells. Among the other analyzed breast tumors, seven showed elevated *TSLC1* CpG island methylation, but to a lesser extent than the three major cases. This might reflect even more increased tumor heterogeneity. The inherent cellular heterogeneity of tissue material affecting the results can be avoided by using microdissection to achieve purer and more homogenous tissue samples, in which very small amounts of tumor cells are sufficient to reliably determinate the methylation status (Kerjean *et al.* 2001).

TSLC1 was identified by Gomyo *et al.* (1999) as a target gene for 11q23.2 deletions, and it is contained in a 700-kb genomic region that completely suppresses tumorigenicity of the A549 human NSCLC cell line (Murakami *et al.* 1998). By functional complementation, it has been determined that most of this suppressive activity localizes to a 100-kb chromosomal subregion (Murakami *et al.* 1998). Kuramochi *et al.* (2001) demonstrated that *TSLC1* silencing is a frequent event in multiple human cancers, and typically occurs due to promoter region hypermethylation. In addition to *TSLC1*, the same 100-kb subregion harbors, for example, the *ALL1*, *EVA1*, *TMPRSS4* and *IL10RA* genes (UCSC database, http://genome.ucsc.edu). All these genes encode proteins that might promote tumorigenesis (Tan *et al.* 1993, Baffa *et al.* 1995, Guttinger *et al.* 1998, Wallrapp *et al.* 2000).

Apparently, based on recent knowledge, Knudson's two-hit hypothesis could now be modified to include epigenetic mechanisms as an additional means to bring about the required two hits for TSG inactivation. Transcriptional silencing through methylation may occur in different combinations with the other known inactivating mechanisms (e.g. intragenic mutations and loss of chromosomal material) (Jones & Laird 1999). First, hypermethylation together with LOH has been shown to result in the silencing of many genes. For instance, *BRCA1*, in which mutations are rarely found in sporadic breast cancers, was found to be hypermethylated in both breast and ovarian tumors informative for LOH (Esteller *et al.* 2000). In breast cancer, both *CDH13* and *FHIT* are silenced by a combination of LOH and methylation (Toyooka *et al.* 2001, Zöchbauer-Müller *et al.*

2001). Second, two transcriptional pathways *per se* can make up the two hits required for gene silencing. This is the case with *RARβ2*, where both biallelic epigenetic inactivation and epigenetic modification combined with LOH occur in breast cancer (Yang *et al.* 2001). Also, in invasive ductal carcinoma (IDC), methylation and increased expression of the negative regulator Snail affecting *CDH1* expression were found to result in gene inactivation (Cheng *et al.* 2001). Interestingly, in IDC, methylation of *CDH1* rarely occurs in combination with LOH, whereas in invasive lobular carcinoma (ILC), all the three mechanisms take place in different combinations (Cheng *et al.* 2001, Droufakou *et al.* 2001). Third, according to Esteller *et al.* (2001b), CpG island promoter hypermethylation can also be considered a mechanism to accomplish 'second hits' for the inactivation of tumor suppressor genes in breast cancer families. In this study, aberrant methylation was never observed in a tumor exhibiting LOH, indicating that in tumors of hereditary origin, methylation does not coincide with allelic loss. On the contrary, if both alleles were present in the tumor and one carried a germline mutation, promoter methylation was frequently found to accomplish the second hit leading to biallelic inactivation of gene expression. Other genes in which hypermethylation has been shown to correlate with a loss of gene expression in breast cancer include *RASSF1A*, (Burbee *et al.* 2001), *NES1* (Li *et al.* 2001), *CDKN2A* (Esteller *et al.* 2001a), *GSTP1* (Esteller *et al.* 2001a), *APC* (Jin et al. 2001), *SRBC* (Xu et al. 2001) and *HIC1* (Nicoll *et al.* 2001).

In human cancers, some methylation patterns are shared by the different tumors types, but some genes are altered as a group in a tumor type-specific manner, probably stressing the importance of the methylation process similarly to the loss of mismatch repair function (Esteller *et al.* 2001a). Both MMR and methylation might influence both critical and non-critical loci, and subsequently, only those interfering with genes essential for genomic integrity would confer selective advantage to tumor growth (Toyota *et al.* 1999, Baylin *et al.* 2000, Costello *et al.* 2000, Esteller *et al.* 2001a).

Taken together, in addition to non-small-cell lung cancer, hepatocellular carcinoma, and pancreatic cancer, *TSLC1* promoter hypermethylation has now also been detected in primary breast cancer. Together with LOH of the other allele, this event could result in gene silencing in at least in 10% (3/30) of breast cancer cases. At present, however, it seems evident that *TSLC1* is not the only target gene harbored in this chromosomal region important for tumor-suppressor activity. Therefore, supportive studies on breast cancer cell lines and microdissected tissue material will be carried out to evaluate further the role of *TSLC1* promoter region CpG hypermethylation in this malignancy.

7 Future directions

Given that all the three studied DSB response genes, *TP53*, *CHK2* and *ATM*, display at least some pathogenic germline mutations in breast cancer patients with familial breast cancer, other DNA damage response genes, such as *NBS1*, *MRE11A*, *BACH1*, *BARD1* and *RAD51* (see chapter 2.5.1.3 and Shinohara *et al*. 1993, Petrini *et al.* 1995, Wu *et al.* 1996, Carney *et al.* 1998, Varon *et al.* 1998, Cantor *et al.* 2001), are also good candidates to harbor mutations related to breast cancer susceptibility. For instance, two germline mutations in *BACH1* have been detected in early-onset breast cancer patients, one of which had a background of familial cancer (Cantor *et al.* 2001). Somatic *BARD1* mutations in combination with loss of the wild-type allele have been reported in breast, ovarian and endometrial tumors (Thai *et al.* 1998). Recently, also germline *BARD1* mutations were discovered in three breast-ovarian cancer families, however the effect of these alterations on protein function remains to be determined (Ghimenti *et al.* 2002).

In contrast to the actual pathogenic mutations, certain variants of these genes might only have a subtle effect on cancer predisposition by themselves. In some cases, however, in combination with other constitutive alterations in genes belonging to the same or some other supplementary biochemical pathway, their cumulative effect could be sufficient to explain the increased breast cancer risk. As a result, less somatic alterations would be required for the malignant transformation of a cell. Consequently, screening for germline mutations in only one or two such genes at a time might not be a sufficiently powerful approach to provide the desired information about the contribution of DSB-related genes to breast cancer. Instead, simultaneous assessments of several genes in large sample sets, combined with high-throughput expression studies, would be required to gain more insight into the putative cumulative effects of rare variants in DNA damage response genes.

Obviously, at present there are also other concurrent efforts to elucidate factors involved in the inherited breast cancer susceptibility in the Finnish population. First, some of the *BRCA1* and *BRCA2* mutation-negative families also included to this study have been selected for genetic linkage studies initiated to identify additional high-penetrance susceptibility genes. As has already been successfully demonstrated (Hedenfalk *et al.* 2001), microarray-based expression analysis will provide an efficient way to cluster breast cancer families into certain smaller subgroups based on the

molecular profiles of the studied tumors. This will greatly facilitate the selection of a more homogenous study population for genetic linkage analysis. Second, as the contribution of certain *ATM* mutations to breast cancer risk was recently confirmed in a large case-control association study (Chenevix-Trench *et al*. 2002), our intention is to perform a survey among Finnish breast cancer patients to see whether any of the *ATM* mutations found in Finnish AT patients have similar effects in our population. Third, due to the availability of the human genome sequence, it will be possible to perform high-throughput somatic mutation searches to trace genes frequently altered in cancer. Consequently, a subset of these genes is also likely to carry cancer-predisposing germline mutations, thus providing a list of candidates to be screened in cancer families and case-control studies (Nathanson *et al.* 2001).

Finally, as the contribution of CpG island hypermethylation to tumorigenesis has been well established (Jones & Laird 1999, Baylin & Herman 2000), it will be interesting to further investigate the role of epigenetic cancer-associated changes in breast cancer. For example, we have expanded our study of *TSLC1* to a larger cohort of breast cancer patients in order to gain more fundamental understanding of the role of this gene in tumorigenesis. Furthermore, new techniques are needed to more efficiently study the phenomenon. For this purpose, a microarray-based strategy to investigate genome-wide CpG island hypermethylation patterns has recently been developed (Huang *et al.* 1999, Yan *et al.* 2001). This method does not only offer an alternative to cDNA microarrays for molecular classification of tumors, but may also have diagnostic potential, as it could be utilized to predict patients' responsiveness to demethylating agents (Yan *et al.* 2001).

8 Concluding remarks

In this study, we investigated the roles of three putative breast cancer predisposing genes (*TP53*, *CHK2* and *ATM*), which are known to be essential in the pathways for cellular signaling and the maintenance of genomic integrity. Germline mutations in these genes are also associated with known cancer syndromes, frequently displaying breast cancer as a major phenotype. Furthermore, to gain more insight into the chromosome 11q21-q24 LOH targets, four candidate genes were screened for intragenic mutations, and five were analyzed for epigenetic inactivation. The observations and conclusions based on the results are:

1. In combination with a previous study (Huusko *et al.* 1999), *TP53* germline mutations were detected in 2.6% (3/108) of the breast cancer families, all showing characteristics of LFS or LFL. The only detected *CHK2* alteration with a putative effect on cancer susceptibility (Ile157Thr) segregated ambiguously with the disease, and it was also present in cancer-free controls. The available functional data, however, suggests that Ile157Thr disturbs the protein function by interfering with the FHA domain-binding ability, and it therefore seems likely that the altered CHK2 in some way promotes cancer formation. Furthermore, compared to the other studied populations, Ile157Thr appears to be markedly enriched in the Finnish population. Thus, the clinical significance of Ile157Thr requires further investigation among Finnish cancer patients.

2. *ATM* germline mutations appear to contribute to a small proportion of the hereditary breast cancer risk, as two distinct *ATM* mutations were found among the three families (1.9%, 3/162) displaying breast cancer. They all originated from the same geographical region as the AT families with the corresponding mutations, providing a preliminary suggestion of a possible founder effect concerning the distribution of these mutations in the Finnish population.

3. The low frequency or absence of somatic intragenic mutations observed among sporadic breast tumors in the putative LOH target genes (*MRE11A*, *PPP2R1B*, *CHK1*, *TSLC1*) residing in chromosome 11q21-q24 led us to assess the role of

promoter region hypermethylation as a mechanism capable of silencing these genes, as well as the *ATM* gene. Of the five genes studied, only *TSLC1* demonstrated involvement of CpG island methylation, which was especially prominent in three tumors. Altogether, one third of the analyzed tumors showed some evidence of *TSLC1* promoter methylation. This suggests that together with LOH, methylation could result in biallelic inactivation of the *TSLC1* gene in breast cancer.

9 References

Aas T, Børresen A-L, Geisler S, Smith-Sørensen B, Johnsen H, Varhaug JE, Akslen LA & Lønning PE (1996) Specific p53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nature Med 2: 811-814.

Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X & Akey CW (2002) Three-dimensional structure of the apoptosome. Implications for assembly, procaspase-9 binding, and activation. Mol Cell 9: 423-432.

Ahn J-Y, Schwarz JK, Piwnica-Worms H & Canman CE (2000) Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizing radiation. Cancer Res 60: 5934-5936.

Alani E, Lee S, Kane MF, Griffith J & Kolodner RD (1997) Saccharomyces cerevisiae MSH2, a mispaired base recognition protein, also recognizes Holliday junctions in DNA. J Mol Biol 265: 289-301.

Ángele S & Hall J (2000) The ATM gene and breast cancer: is it really a risk factor? Mutat Res 462: 167-178.

Athma P, Rappaport R & Swift M (1996) Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. Cancer Genet Cytogenet 92: 130-134.

Baffa R, Negrini M, Schichman SA, Huebner K & Croce CM (1995) Involvement of the ALL-1 gene in a solid tumor. Proc Natl Acad Sci USA 92: 4922-4926.

Banin S, Moyal L, Shieh S-Y, Taya Y, Anderson CW, Chessa L, Smorodinsky NI, Prives C, Reiss Y, Shiloh Y & Ziv Y (1998) Enhanced phosphorylation of p53 by ATM in response to DNA damage. Science 281: 1674-1677.

Barlow C, Dennery PA, Shigenaga MK, Smith MA, Morrow JD, Roberts LJ 2nd, Wynshaw-Boris A & Levine RL (1999) Loss of ataxia-telangiectasia gene product causes oxidative damage in target organs. Proc Natl Acad Sci USA 96: 9915-9919.

Barlow C, Ribaut-Barassin C, Zwingman TA, Pope AJ, Brown KJ, Owens JW, Larson D, Harrington EA, Haeberle A-M, Mariani J, Eckhaus M, Herrup K, Bailly Y & Wynshaw-Boris A (2000) ATM is a cytoplasmic protein in mouse brain required to prevent lysosomal accumulation. Proc Natl Acad Sci USA 97: 871-876.

Bartek J & Lukas J (2001) Mammalian G1- and S-phase checkpoints in response to DNA damage. Curr Opin Cell Biol 13: 738-747.

Baskaran R, Wood LD, Whitaker LL, Canman CE, Morgan SE, Xu Y, Barlow C, Baltimore D, Wynshaw-Boris A, Kastan MB & Wang JYJ (1997) Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. Nature 387: 516-519.

Battaglia F, Scambia G, Rossi S, Beneditti Panici P, Bellantone R, Polizzi G, Querzoli P, Negrini R, Iacobelli S, Crucitti F & Mancuso S (1988) Epidermal growth factor receptor in human breast cancer: correlation with steroid hormone receptors and axillary lymph node involvement. Eur J Cancer Clin Oncol 24: 1685-1690.

Bay J-O, Grancho M, Pernin D, Presneau N, Rio P, Tchirkov A, Uhrhammer N, Verrelle P, Gatti RA & Bignon Y-J (1998) No evidence for constitutional ATM mutation in breast/gastric cancer families. Int J Oncol 12: 1385-1390.

Baylin SB, Belinsky SA & Herman JG (2000) Aberrant methylation of gene promoters in cancer-concepts, misconcepts and promise. J Natl Cancer Inst 92: 1460-1461.

Baylin SB & Herman JG (2000) DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet 16: 168-174.

Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PEM, Rubinstein WS, Myers EN, Richard 3rd CW, Cornelisse CJ, Devilee P & Devlin B (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287: 848-851.

Bebb DG, Yu Z, Chen J, Telatar M, Gelmon K, Phillips N, Gatti RA & Glickman BW (1999) Absence of mutations in the ATM gene in forty-seven cases of sporadic breast cancer. Br J Cancer 80: 1979-1981.

Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DCR, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE & Haber DA (1999) Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science 286: 2528-2531.

Bennett RJ, Keck JL & Wang JC (1999) Binding specificity determines polarity of DNA unwinding by the Sgs1 protein of S.cerevisiae. J Mol Biol 289: 235-248.

Berg JW & Hutter RVP (1995) Breast cancer. Cancer 75: 257-269.

Berns EMJJ, Foekens JA, Vossen R, Look MP, Devilee P, Henzen-Logmans SC, van Staveren IL, van Putten WLJ, Inganäs M, Meijer-van Gelder ME, Cornelisse C, Claassen CJC, Portengen H, Bakker B & Klijn JGM (2000) Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer. Cancer Res 60: 2155-2162.

Berns EMJJ, van Staveren IL, Look MP, Smid M, Klijn JGM & Foekens JA (1998) Mutations in residues of TP53 that directly contact DNA predict poor outcome in human primary breast cancer. Br J Cancer 77: 1130-1136.

Bertoni F, Codegoni AM, Furlan D, Tibiletti MG, Capella C & Broggini M (1999) CHK1 frameshift mutations in genetically unstable colorectal and endometrial cancers. Genes Chrom Cancer 26: 176-180.

Bestor TH (1998) Methylation meets acetylation. Nature 393: 311-312.

Bhattacharyya NP, Skandalis A, Ganesh A, Groden J & Meuth M (1994) Mutator phenotypes in human colorectal carcinoma cell lines. Proc Natl Acad Sci USA 91: 6319-6323.

Bièche I & Lidereau R (1995) Genetic alterations in breast cancer. Genes Chrom Cancer 14: 227-251.

Birch JM, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM, Harris M, Morris Jones PH, Binchy A, Crowther D, Craft AW, Eden OB, Evans DGR, Thompson E, Mann JR, Martin J, Mitchell ELD & Santibáñes-Koref MF (1994) Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. Cancer Res 54: 1298-1304.

Bland KI, Menck HR, Scott-Conner CEH, Morrow M, Winchester DJ & Winchester DP (1998) The national cancer data base 10-year survey of breast carcinoma treatment at hospitals in the United States. Cancer 83: 1262-1273.

Boardman LA, Thibodeau SN, Schaid DJ, Lindor NM, McDonnell SK, Burgart LJ, Ahlquist DA, Podratz KC, Pittelkow M & Hartmann LC (1998) Increased risk for cancer in patients with the Peutz-Jehgers syndrome. Ann Intern Med 128: 896-899.

Bochar DA, Wang L, Beniya H, Kinev A, Xue Y, Lane WS, Wang W, Kashanchi F & Shiekhatter R (2000) BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. Cell 102: 257-265.

Bork P, Hofman K, Bucher P, Neuwald AF, Altschul SF & Koonin EV (1997) A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. FASEB J 11: 68-76.

Børresen A-L, Andersen TI, Eyfjord JE, Cornelis RS, Thorlacius S, Borg Å, Johansson U, Theillet C, Scherneck S & Hartman S (1995) TP53 mutations and breast cancer prognosis: particularly poor survival rates for cases with mutations in the zinc-binding domains. Genes Chrom Cancer 14: 71-75.

Børresen A-L, Andersen TI, Garber J, Barbier-Piraux N, Thorlacius S, Eyfjörd J, Ottestad L, Smith-Sørensen B, Hovig E, Malkin D & Friend SH (1992) Screening for germ line TP53 mutations in breast cancer patients. Cancer Res 52: 3234-3236.

Bougeard G, Limacher J-M, Martin C, Charbonnier F, Killian A, Delattre O, Longy M, Jonveaux P, Fricker J-P, Stoppa-Lyonnet D, Flaman J-M & Frébourg T (2001) Detection of 11 germline inactivating TP53 mutations and absence of TP63 and HCHK2 mutations in 17 French families with Li-Fraumeni or Li-Fraumeni-like syndrome. J Med Genet 38: 253-257.

Breast Cancer Linkage Consortium (1999) Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst 91: 1310-1316.

Broeks A, Urbanus JHM, Floore AN, Dahler EC, Klijn JGM, Rutgers EJT, Devilee P, Russle NS, van Leeuwen FE & van't Veer LJ (2000) ATM-heterozygous germline mutations contribute to breast cancer-susceptibility. Am J Hum Genet 66: 494-500.

Brown KD, Ziv Y, Sadanandan SN, Chessa L, Collins FS, Shiloh Y & Tagle DA (1997) The ataxia-telangiectasia gene product, a constitutively expressed nuclear protein that is not up-regulated following genome damage. Proc Natl Acad Sci USA 94: 1840-1845.

Budhram-Mahadeo V, Morris PJ, Smith MD, Midgeley CA, Boxer LM & Latchman DS (1999) p53 suppresses the activation of the Bcl-2 promoter by the Brn-3a POU family transcription factor. J Biol Chem 274: 15237-15244.

Burbee DG, Forgacs E, Zöchbauer-Müller S, Shivakumar L, Fong K, Gao B, Randle D, Kondo M, Virmani A, Bader S, Sekido Y, Latif F, Milchgrub S, Toyooka S, Gazdar AF, Lerman MI, Zabarovsky E, White M & Minna J (2001) Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. J Natl Cancer Inst 93: 691-699.

Buscemi G, Savio C, Zannini L, Micciché F, Masnada D, Nakanishi M, Tauchi H, Komatsu K, Mizutani S, Khanna KK, Chen P, Concannon P, Chessa L & Delia D (2001) Chk2 activation dependence on Nbs1 after DNA damage. Mol Cell Biol 21: 5214-5222.

Calin GA, di Iasio MG, Caprini E, Vorechovsky I, Natali PG, Sozzi G, Croce CM, Barbanti-Brodano G, Russo G & Negrini M (2000) Low frequency of alterations of the α (PPP2R1A) and β (PPP2R1B) isoforms of the subunit A of the serine-theonine phosphatase 2A in human neoplasms. Oncogene 19: 1191-1195.

Canman CE, Lim D-S, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, Appella E, Kastan MB & Siciliano JD (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science 281: 1677-1679.

Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DCR, Sgroi DC, Lane WS, Haber DA & Livingston DM (2001) BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell 105: 149-160.

Cao J, Cai X, Zheng L, Geng L, Shi Z, Pao CC & Zheng S (1997) Characterization of colorectal-cancer-related cDNA clones obtained by subtractive hybridization screening. J Cancer Res Clin Oncol 123: 447-451.

Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates JR III, Hays L, Morgan WF & Petrini JHJ (1998) The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. Cell 93: 477-486.

Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, Murphree AL, Strong LC & White RL (1983) Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 305: 779-784.

Chehab NH, Malikzay A, Appel M & Halazonetis TD (2000) Chk2/hCds1 functions as a DNA damage checkpoint in G1 by stabilizing p53. Genes Dev 14: 278-288.

CHEK2-Breast Cancer Consortium (2002) Low-penetrance susceptibility to breast cancer due to CHEK2*1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nature Genet 31: 55-59.

Chen G & Lee EY-HP (1996) The product of the ATM gene is a 370-kDa nuclear phosphoprotein. J Biol Chem 271: 33693-33697.

Chen J (2000) Ataxia telagiectasia-related protein is involved in the phosphorylation of BRCA1 following deoxyribonucleic acid damage. Cancer Res 60: 5037-5039.

Chen J, Giesler Birkholz G, Lindblom P, Rubio C & Lindblom A (1998) The role of ataxia-telangiectasia heterozygotes in familial breast cancer. Cancer Res 58: 1376-1379.

Chen PC, Lavin MF & Kidson C (1978) Identification of ataxia telangiectasia heterozygotes, a cancer prone population. Nature 274: 484-486.

Chenevix-Trench G, Spurdle AB, Gatei M, Kelly H, Marsh A, Chen X, Donn K, Cummings M, Nyholt D, Jenkins MA, Scott C, Pupo GM, Dörk T, Bendix R, Kirk J, Tucker K, McCredie MRE, Hopper JL, Sambrook J, Mann GJ & Khanna KK (2002) Dominant negative ATM mutations in breast cancer families. J Natl Cancer Inst 94: 205-215.

Cheng C-W, Wu P-E, Yu J-C, Huang C-S, Yue C-T, Wu C-W & Shen C-Y (2001) Mechanisms of inactivation of E-cadherin in breast carcinoma: modification of the two-hit hypothesis of tumor suppressor gene. Oncogene 20: 3814-3823.

Cho Y, Gorina S, Jeffrey PD & Pavletich NP (1994) Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science 265: 346-355.

Chong MJ, Murray MR, Gosnick EC, Russel HRC, Srinivasan A, Kapsetaki M, Korsmeyer SJ & McKinnon PJ (2000) Atm and Bax cooperate in ionizing radiation-induced apoptosis in the central nervous system. Proc Natl Acad Sci USA 97: 889-894.

Clark SJ, Harrison J, Paul CL & Frommer M (1994) High sensitivity mapping of methylated cytosines. Nucl Acids Res 22: 2990-2997.

Claus EB, Schildkraut JM, Thompson WD & Risch NJ (1996) The genetic attributable risk of breast and ovarian cancer. Cancer 77: 2318-2324.

Cleaver JE, Karplus K, Kashani-Sabet M & Limoli CL (2001) Nucleotide excision repair "a legacy of creativity". Mutat Res 485: 23-36.

Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G & Slamon DJ (1999) Multinational study of the efficacy and safety of humanized anti-HER-2 monoclonal antibody in women who have HER-2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17: 2639-2648.

Cooper PK, Nouspikel T, Clarkson SG & Leadon SA (1997) Defective transcription-coupled repair of oxidative base damage in Cockayne syndrome patients from XP group G. Science 275: 990-993.

Cortez D, Guntuku S, Qin J & Elledge SJ (2001) ATR and ATRIP: partners in checkpoint signalling. Science 294: 1713-1716.

Cortez D, Wang Y, Qin J & Elledge S (1999) Requirement of ATM-dependent phosphorylation of Brca1 in the DNA damage response to double-strand breaks. Science 286: 1162-1166.

Costanzo V, Robertson K, Ying CY, Kim E, Avvedimento E, Gottesman M, Grieco D & Gautier J (2000) Reconsitution of an ATM-dependent checkpoint that inhibits chromosomal DNA replication following DNA damage. Mol Cell 6: 649-659.

Costello JF, Frühwald MC, Smiraglia DJ, Rush LJ, Robertson GP, Gao X, Wright FA, Feramisco JD, Peltomäki P, Lang JC, Schuller DE, Yu L, Bloomfield CD, Caligiuri MA, Yates A, Nishikawa R, Su Huang H-J, Petrelli NJ, Zhang X, O'Dorisio MS, Held WA, Cavenee WK & Plass C (2000) Aberrant CpG-island methylation has non-random and tumor-type-specific patterns. Nature Genet 25: 132-138.

Couch FJ & Weber BL (1998) Breast cancer. In: Vogelstein B & Kinzler KW (eds) The genetic basis of human cancer: 537-563. McGraw-Hill, New York.

Courjal F & Theillet C (1997) Comparative genomic hybridization analysis of breast tumors with predetermined profiles of DNA amplification. Cancer Res 57: 4368-4377.

Cui J, Antoniou AC, Dite GS, Southey MC, Venter DJ, Easton DF, Giles GG, McCredie MRE & Hopper JL (2001) After BRCA1 and BRCA2 - what next? Multifactorial segregation analyses of three-generation, population-based Australian families affected by female breast cancer. Am J Hum Genet 68: 420-431.

Davies AA, Masson J-Y, Mcllwraith MJ, Stasiak AZ, Stasiak A, Venkitaraman AR & West SC (2001) Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. Mol Cell 7: 273-282.

Deng X, Ito T, Carr B, Mumby M & May Jr WS (1998) Reversible phosphorylation of Bcl2 following interleukin 3 or bryostatin 1 is mediated by direct interaction with protein phosphatase 2A. J Biol Chem 273: 34157-34163.

de Laat WL, Jaspers NGJ & Hoeijmakers JHJ (1999) Molecular mechanism of nucleotide excision repair. Genes Dev 13: 768-785.
Dobrovic A & Simpfendorfer D (1997) Methylation of the BRCA1 gene in sporadic breast cancer. Cancer Res 57: 3347-3350.
Dowsett M (1998) Improved prognosis for biomarkers in breast cancer. Lancet 351: 1753-1754.
Droufakou S, Deshmane V, Roylance R, Hanby A, Tomlinson I & Hart IR (2001) Multiple ways of silencing E-cadherin gene expression in lobular carcinoma of the breast. Int J Cancer 92: 404-408.
Duffy MJ (2001) Biochemical markers in breast cancer: which ones are clinically useful? Clin Biochem 34: 347-352.
Duffy MJ, Maguire TM, McDermott EW & O'Higgins N (1999) Urokinase plasminogen activator: a prognostic marker in multiple types of cancer. J Surg Oncol 71: 130-135.
Dunning AM, Healey CS, Pharoah PDP, Teare MD, Ponder BAJ & Easton DF (1999) A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers & Prev 8: 843-854.
Dunphy WG (1994) The decision to enter mitosis. Trends Cell Biol 4: 202-207.
Dörk T, Bendix R, Bremer M, Rades D, Klöpper K, Nicke M, Skawran B, Hector A, Yamini P, Steinmann D, Weise S, Stuhrmann M & Karstens JH (2001) Spectrum of ATM gene mutations in a hospital-based series of unselected breast cancer patients. Cancer Res 61: 7608-7615.
Easton DF, Bishop DT, Ford D, Crockford GP & the Breast Cancer Linkage Consortium (1993) Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. Am J Hum Genet 52: 678-701.
Ejima Y & Sasaki MS (1998) Mutations of the ATM gene detected in Japanese ataxia-telangiectasia patients: possible preponderance of the two founder mutations 4612del165 and 7883del5. Hum Genet 102: 403-408.
Elston CW, Ellis IO & Pinder SE (1999) Pathological prognostic factors in breast cancer. Crit Rev Oncol Hematol 31: 209-223.
Eng C, Schneider K, Fraumeni JF Jr & Li FP (1997) Third international workshop on collaborative interdisciplinary studies of p53 and other predisposing genes in Li-Fraumeni syndrome. Cancer Epidemiol Biomarkers Prev 6: 379-383.
Esteller M (2000) Epigenetic lesions causing genetic lesions in human cancer: promoter hypermethylation of DNA repair genes. Eur J Cancer 36: 2294-2300.
Esteller M, Corn PG, Baylin SB & Herman JG (2001a) A gene hypermethylation profile of human cancer. Cancer Res 61: 3225-3229.
Esteller M, Fraga MF, Guo M, Garcia-Foncillas J, Hedenfalk I, Godwin AK, Trojan J, Vaurs-Barrière C, Bignon Y-J, Ramus S, Benitez J, Caldes T, Akiyama Y, Yuasa Y, Launonen V, Canal MJ, Rodriguez R, Capella G, Peinado MA, Borg A, Aaltonen LA, Ponder BA, Baylin SB & Herman JG (2001b) DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. Hum Mol Genet 10: 3001-3007.
Esteller M, Hamilton SR, Burger PC, Baylin SB & Herman JG (1999) Inactivation of the DNA repair gene O^6-methylguanine-DNA methyltrasferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res 59: 793-797.
Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M, Baylin SJ & Herman JG (2000) Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 92: 564-569.
Falck J, Lukas C, Protopopova M, Lukas J, Selivanova G & Bartek J (2001a) Functional impact of concomitant versus alternative defects in the Chk2-p53 tumor suppressor pathway. Oncogene 20: 5503-5510.
Falck J, Mailand N, Syljuåsen RG, Bartek J & Lukas J (2001b) The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature 410: 842-847.
Falck J, Petrini JHJ, Williams BR, Lukas J & Bartek J (2002) The DNA damage-dependent intra-S phase checkpoint is regulated by parallel pathways. Nature Genet 30: 290-294.
Fearon ER (1998) Tumor suppressor genes. In: Vogelstein B & Kinzler KW (eds) The genetic basis of human cancer: 229-236. McGraw-Hill, New York.

Fearon ER & Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61: 759-767.
Finnish Cancer Registry. Institute for Statistical and Epidemiological Cancer Research (2002) Preliminary incidence figures for 1999. Available from: http://www.cancerregistry.fi.
FitzGerald MG, Bean JM, Hegde SR, Unsal H, MacDonald DJ, Harkin DP, Finkelstein DM, Isselbacher KJ & Haber DA (1997) Heterozygous ATM mutations do not contribute to early onset of breast cancer. Nature Genet 15: 307-310.
Flaggs G, Plug AW, Dunks KM, Mundt KE, Ford JC, Quiggle MRE, Taylor EM, Westphal CH, Ashley T, Hoekstra MF & Carr AM (1997) Atm-dependent interactions of a mammalian Chk1 homolog with meiotic chromosomes. Curr Biol 7: 977-986.
Foekens JA, Schmitt M, van Putten WLJ, Peters HA, Kramer MD, Jänicke F & Klijn JGM (1994) Plasminogen activator inhibitor-1 and prognosis in primary breast cancer. J Clin Oncol 12: 1648-1658.
Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE & the Breast Cancer Linkage Consortium (1994) Risks of cancer in BRCA1-mutation carriers. Lancet 343: 692-695.
Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkadottir R, Eyfjord J, Lynch H, Ponder BAJ, Gayther SA, Birch JM, Lindblom A, Stoppa-Lyonnet D, Bignon Y, Borg A, Hamann U, Haites N, Scott RJ, Maugard CM, Vasen H, Seitz S, Cannon-Albright LA, Schofield A, Zelada-Hedman M & the Breast Cancer Linkage Consortium (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62: 676-689.
Forozan F, Mahlamäki EH, Monni O, Chen Y, Veldman R, Jiang Y, Gooden GC, Ethier SP, Kallioniemi A & Kallioniemi O-P (2000) Comparative genomic hybridization analysis of 38 breast cancer cell lines: a basis for interpreting complementary DNA microarray data. Cancer Res 60: 4519-4525.
Frommer M, McDonald LE, Millar DS, Collis CM, Watt F, Grigg GW, Molloy PL & Paul CL (1992) A genomic sequencing protocol that yields a positive display of 5-methycytosine residues in individual DNA strands. Proc Natl Acad Sci USA 89: 1827-1831.
Fukuda T, Sumiyoshi T, Takahashi M, Kataoka T, Asahara T, Inui H, Watatani M, Yasutomi M, Kamada N & Miyagawa K (2001) Alterations of the double-strand break repair gene MRE11 in cancer. Cancer Res 61: 23-26.
Fukuhara H, Kuramochi M, Nobukuni T, Fukami T, Saino M, Maruyama T, Nomura S, Sekiya T & Murakami Y (2001) Isolation of the TSLL1 and TSLL2 genes, members of the tumor suppressor TSLC1 gene family encoding transmembrane proteins. Oncogene 20: 5401-5407.
Futaki M & Liu JM (2001) Chromosomal breakage syndromes and the BRCA1 genome surveillance complex. Trens Mol Med 7: 560-565.
Ganguly A, Rock MJ & Prockop DJ (1993) Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: Evidence for solvent-induced bends in DNA heteroduplexes. Proc Natl Acad Scie USA 90: 10325-10329.
Gatei M, Zhou B-B, Hobson K, Scott S, Young D & Khanna KK (2001) Ataxia telangiectasia mutated (ATM) kinase and ATM and Rad3 related kinase mediate phosphorylation of Brca1 at distinct and overlapping sites. J Biol Chem 276: 17276-17280.
Gatti RA (1998) Ataxia-telangiectasia. In: Vogelstein B & Kinzler KW (eds) The genetic basis of human cancer: 275-300. McGraw-Hill, New York.
Geisler S, L Lønning PE, Aas T, Johnsen H, Fluge Ø, Haugen DF, Lillehaug JR, Akslen LA & Børresen-Dale A-L (2001) Influence of TP53 gene alterations and c-erbB-2 expression on the response to treatment with doxorubicin in locally advanced breast cancer. Cancer Res 61: 2505-2512.
Gelman A, Carlin JB, Stern HS & Rubin DB (1995) Bayesian data analysis: 3-25, 48-51. Chapman & Hall, London.
Gentile M, Bergman Jungeström M, Olsen KE, Söderkvist P & Wingren S (1999) p53 and survival in early onset breast cancer: analysis of gene mutations, loss of heterozygosity and protein accumulation. Eur J Cancer 35: 1202-1207.

Geoffroy-Perez B, Janin N, Ossian K, Laugé A, Croquette M-F, Griscelli C, Debré M, Bressac-de-Paillerets B, Aurias A, Stoppa-Lyonnet D & Andrieu N (2001) Cancer risk in heterozygotes for ataxia-telangiectasia. Int J Cancer 93: 288-293.

Ghimenti C, Sensi E, Presciuttini S, Brunetti IM, Conte P, Bevilacqua G & Caligo MA (2002) Germline mutations of the BRCA1-associated ring domain (BARD1) gene in breast and breast/ovarian families negative for BRCA1 and BRCA2 alterations. Genes Chrom Cancer 33: 235-242.

Gilad S, Khosravi R, Shkedy D, Uziel T, Ziv Y, Savitsky K, Rotman G, Smith S, Chessa L, Jorgensen TJ, Harnik R, Frydman M, Sanal O, Portnoi S, Goldwicz Z, Jaspers NGJ, Gatti RA, Lenoir G, Lavin MF, Tatsumi K, Wegner RD, Shiloh Y & Bar-Shira A (1996) Predominance of null mutations in ataxia-telangiectasia. Hum Mol Genet 5: 433-439.

Goedecke W, Eijpe M, Offenberg HH, van Aalderen M & Heyting C (1999) Mre11 and Ku70 interact in somatic cells, but are differentially expressed in early meiosis. Nature Genet 23: 194-198.

Gomyo H, Arai Y, Tanigami A, Murakami Y, Hattori M, Hosoda F, Arai K, Aikawa Y, Tsuda H, Hirohashi S, Asakawa S, Shimizu N, Soeda E, Sakaki Y & Ohki M (1999) A 2-Mb sequence-ready contig map and a novel immunoglobulin superfamily gene IGSF4 in the LOH region of chromosome 11q23.2. Genomics 62: 139-146.

Gross A, McDonnell JM & Korsmeyer SJ (1999) BCL-2 family members and the mithocondria in apoptosis. Genes Dev 13: 1899-1911.

Groves MR, Hanlon N, Turowski P, Hemmings BA & Barford D (1999) The structure of the protein phosphatase 2A PR65/A subunit reveals the conformation of its 15 tandemly repeated HEAT motifs. Cell 96: 99-110.

Guttinger M, Sutti F, Panigada M, Porcellini S, Merati B, Mariani M, Teesalu T, Consalez GG & Grassi F (1998) Epithelian V-like antigen (EVA), a novel member of the immunoglobulin superfamily, expressed in embryonic epithelia with a potential role as homotypic adhesion molecule in thymus histogenesis. J Cell Biol 141: 1061-1071.

Haber J (2000) Partners and pathways: repairing a double-strand break. Trends Genet 16: 259-264.

Hampton GM, Mannermaa A, Winqvist R, Alavaikko M, Blanco G, Taskinen PJ, Kiviniemi H, Newsham I, Cavenee WK & Evans GA (1994) Loss of heterozygosity in sporadic human breast carcinoma: a common region between 11q22 and 11q23.3. Cancer Res 54: 4586-4589.

Hande MP, Balajee AS, Tchirkov A, Wynshaw-Boris A & Lansdrop PM (2001) Extra-chromosomal telomeric DNA in cells from Atm-/- mice and patients with ataxia-telangiectasia. Hum Mol Genet 10: 519-528.

Harfe BD & Jinks-Robertson S (2000) DNA mismatch repair and genetic instability. Annu Rev Genet 34: 359-399.

Haruki N, Saito H, Tatematsu Y, Konishi H, Harano T, Masuda A, Osada H, Fujii Y & Takahashi T (2000) Histological type-selective, tumor-predominant expression of a novel CHK1 isoform and infrequent in vivo somatic CHK2 mutation in small cell lung cancer. Cancer Res 60: 4689-4692.

Hedenfalck I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, Meltzer P, Gusterson B, Esteller M, Kallioniemi O-P, Wilfond B, Borg Å & Trent J (2001) Gene-expression profiles in hereditary breast cancer. N Engl J Med 344: 539-548.

Hemmings BA, Adams-Pearson C, Maurer F, Müller P, Goris J, Merlevede W, Hofsteenge J & Stone SR (1990) α- and β-forms of the 65-kDa subunit of protein phosphatase 2A have a similar 39 amino acid repeating structure. Biochemistry 29: 3166-3173.

Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, Bignell G, Warren W, Aminoff M, Höglund P, Järvinen H, Kristo P, Pelin K, Ridanpää M, Salovaara R, Toro T, Bodmer W, Olschwang S, Olsen AS, Stratton MR, de la Chapelle A & Aaltonen LA (1998) A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 391: 184-187.

Hemminki A, Tomlinson I, Markie D, Järvinen H, Sistonen P, Björkvist A-M, Knuutila S, Salovaara R, Bodmer W, Shibata D, de la Chapelle A & Aaltonen LA (1997) Localization of a susceptibility locus for Peutz-Jehgers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. Nature Genet 15: 87-90.

Hengartner MO (2000) The biochemistry of apoptosis. Nature 407: 770-776.

Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa J-PJ, Markowitz S, Willson JKV, Hamilton SR, Kinzler KW, Kane MF, Kolodner RD, Vogelstein B, Kunkel TA & Baylin SB (1998) Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA 95: 6870-6875.

Herzog K-H, Chong MJ, Kapsetaki M, Morgan JI & McKinnon PJ (1998) Requirement for Atm in ionizing radiation-induced cell death in the developing central nervous system. Science 280: 1089-1091.

Hinds PW, Finlay CA, Quartin RS, Baker SJ, Fearon ER, Vogelstein B & Levine AJ (1990) Mutant p53 DNA clones from human colon carcinomas cooperate with ras in transforming primary rat cells: a comparison of the 'Hot Spot' mutant phenotypes. Cell Growth Differ 1: 571-580.

Hirao A, Kong Y-Y, Matsuoka S, Wakeham A, Ruland J, Yoshida H, Liu D, Elledge SJ & Mak TW (2000) DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science 287: 1824-1827.

Hoeijmakers JHJ (1993a) Nucleotide excision repair I: from E.coli to yeast. Trends Genet 9: 173-177.

Hoeijmakers JHJ (1993b) Nucleotide excision repair II: from yeast to mammals. Trends Genet 9: 211-217.

Hoeijmakers JHJ (2001) Genome maintenance mechanisms for preventing cancer. Nature 411: 366-374.

Hofmann W-K, Miller CW, Tsukasaki K, Tavor S, Ikezoe T, Hoelzer D, Takeuchi S & Koeffler HP (2001) Mutation analysis of the DNA-damage checkpoint gene CHK2 in myelodysplastic syndromes and acute myeloid leukemias. Leuk Res 25: 333-338.

Howat JMT, Barnes DM, Harris M & Swindell R (1983) The association of cytosol oestrogen and progesterone receptors with histological features of breast cancer and early recurrence of disease. Br J Cancer 47: 629-640.

Huang TH-M, Perry MR & Laux DE (1999) Methylation profiling of CpG islands in human breast cancer cells. Hum Mol Genet 8: 459-470.

Huusko P, Castrén K, Launonen V, Soini Y, Pääkkönen K, Leisti J, Vähäkangas K & Winqvist R (1999) Germ-line TP53 mutations in Finnish cancer families exhibiting features of the Li-Fraumeni syndrome and negative for BRCA1 and BRCA2. Cancer Genet Cytogenet 112: 9-14.

Huusko P, Pääkkönen K, Launonen V, Pöyhönen M, Blanco G, Kauppila A, Puistola U, Kiviniemi H, Kujala M, Leisti J & Winqvist R (1998) Evidence of founder mutations in Finnish BRCA1 and BRCA2 families. Am J Hum Genet 62: 1544-1548.

Ino Y, Wahrer DCR, Bell DW, Haber DA & Louis DN (2000) Mutation analysis of the hCHK2 gene in primary human malignant gliomas. Neurogenetics 3: 45-46.

Inskip HM, Kinlen LJ, Taylor AMR, Woods CG & Arlett CF (1999) Risk of breast cancer and other cancers in heterozygotes for ataxia-telangiectasia. Br J Cancer 79: 1304-1307.

International SNP Map Working Group (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 409: 928-933.

Irizarry K, Kustanovich V, Li C, Brown N, Nelson S, Wong W & Lee CJ (2000) Genome-wide analysis of single-nucleotide polymorphisms in human expressed sequences. Nature Genet 26: 233-236.

Isola JJ, Kallioniemi O-P, Chu LW, Fuqua SAW, Hilsenbeck SG, Osborne CK & Waldman FM (1995) Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. Am J Pathol 147: 905-911.

Izatt L, Greenman J, Hodgson S, Ellis D, Watts S, Scott G, Jacobs C, Liebmann R, Zvelebil MJ, Mathew C & Solomon E (1999) Identification of germline missense mutations and rare allelic variants in the ATM gene in early-onset breast cancer. Genes Chrom Cancer 26: 286-294.

Jackson-Grusby L, Beard C, Possemato R, Tudor M, Fambrough D, Csankovski G, Dausman J, Lee P, Wilson C, Lander E & Jaenisch R (2001) Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. Nature Genet 27: 31-39.

Janin N, Andrieu N, Ossian K, Laugé A, Croquette M-F, Griscelli C, Debré M, Bressac-de-Paillerets B, Aurias A & Stoppa-Lyonnet D (1999) Breast cancer risk in ataxia telangiectasia (AT) heterozygotes: haplotype study in French AT families. Br J Cancer 80: 1042-1045.

Janssens V & Goris J (2001) Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. Biochem J 353: 417-439.
Jin Z, Tamura G, Tsuchiya T, Sakata K, Kashiwaba M, Osakabe M & Motoyama T (2001) Adenomatous polyposis coli (APC) gene promoter hypermethylation in primary breast cancers. Br J Cancer 85: 69-73.
Jones PA & Laird PW (1999) Cancer epigenetics comes of age. Nature Genet 21: 163-167.
Jones PL, Veenstra GJC, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J & Wolffe AP (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nature Genet 19: 187-191.
Kainu T, Juo S-HH, Desper R, Schäffer AA, Gillanders E, Rozenblum E, Freas-Lutz D, Weaver D, Stephan D, Bailey-Wilson J, Kallioniemi O-P (Group 1); Tirkkonen M, Syrjäkoski K, Kuukasjärvi T, Koivisto P, Karhu R, Holli K (Group 2); Arason A, Johannesdottir G, Bergthorsson JT, Johannsdottir H, Egilsson V, Barkardottir RB (Group 3); Johannsson O, Haraldsson K, Sandberg T, Holmberg E, Grönberg H, Olsson H, Borg Å (Group 4); Vehmanen P, Eerola H, Heikkilä P, Pyrhönen S & Nevanlinna H (Group 5) (2000) Somatic deletions in hereditary breast cancers implicate 13q21 as a putative novel breast cancer susceptibility locus. Proc Natl Acad Sci USA 97: 9603-9608.
Kallioniemi A, Kallioniemi O-P, Sudar D, Rutovitz D, Gray JW, Waldman F & Pinkel D (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258: 818-821.
Karran P (2000) DNA double strand break repair in mammalian cells. Curr Opin Genet Dev 10: 144-150.
Kastan MB & Lim DS (2000) The many substrates and functions of ATM. Nat Rev Mol Cell Biol 1: 179-186.
Kerangueven F, Eisinger F, Noguchi T, Allione F, Wargniez V, Eng C, Padberg G, Theillet C, Jacquemier J, Longy M, Sobol H & Birnbaum D (1997b) Loss of heterozygosity in human breast carcinomas in ataxia telangiectasia, Cowden disease and BRCA1 gene regions. Oncogene 14: 339-347.
Kerangueven F, Noguchi T, Coulier F, Allione F, Wargniez V, Simony-Lafontaine J, Longy M, Jacquemier J, Sobol H, Eisinger F & Birnbaum D (1997a) Genome-wide search for loss of heterozygosity shows extensive genetic diversity of human breast carcinomas. Cancer Res 57: 5469-5474.
Kerjean A, Vieillefond A, Thiounn N, Sibony M, Jeanpierre M & Jouannet P (2001) Bisulfite genomic sequencing of microdissected cells. Nucleic Acids Res 29: e106.
Khanna KK (2000) Cancer risk and the ATM gene: a continuing debate. J Natl Cancer Inst 92: 795-802.
Khanna KK & Jackson SP (2001) DNA double-strand breaks: signaling, repair and the cancer connection. Nature Genet 27: 247-254.
Kim S-T, Xu B & Kastan MB (2002) Involvement of the cohesin protein, Smc1, in Atm-dependent and independent responses to DNA damage. Genes Dev 16: 560-570.
Kimura K, Shinmura K, Yoshimura K, Shimizu K, Katai H, Beppu Y, Moriya H & Yokota J (2000) Absence of germline CHK2 mutations in familial gastric cancer. Jpn J Cancer Res 91: 875-879.
Kinoshita N, Yamano H, Niwa H, Yoshida T & Yanagida M (1993) Negative regulation of mitosis by the fission yeast protein phosphatase ppa2. Genes Dev 7: 1059-1071.
Kinzler KW & Vogelstein B (1996) Lessons from hereditary colorectal cancer. Cell 87: 159-170.
Kinzler KW & Vogelstein B (1997) Gatekeepers and caretakers. Nature 386: 761-763.
Kleihues P, Schäuble B, zur Hausen A, Estève J & Ohgaki H (1997) Tumors associated with p53 germline mutations: a synopsis of 91 families. Am J Pathol 150: 1-13.
Knudson AG (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68: 820-823.
Knudson AG (1978) Retinoblastoma: a prototypic hereditary neoplasm. Semin Oncol 5: 57-60.
Koi M, Morita H, Yamada H, Satoh H, Barrett JC & Oshimura M (1989) Normal human chromosome 11 suppresses tumorigenicity of human cervical tumor cell line SiHa. Mol Carcinogen 2: 12-21.

Kollias J, Man S, Marafie M, Carpenter K, Pinder S, Ellis IO, Blamey RW, Cross G & Brook JD (2000) Loss of heterozygosity in bilateral breast cancer. Breast Cancer Res Treat 64: 241-251.

Koonin EV, Altschul SF & Bork P (1996) Functional motifs. Nature Genet 13: 266-268.

Koreth J, Bakkenist CJ, Larin Z, Hunt NCA, James MR & McGee JO (1999) 11q23.1 and 11q25-qter YACs suppress tumour growth in vivo. Oncogene 18: 1157-1164.

Koreth J, Bakkenist CJ & McGee JO (1997) Allelic deletions at chromosome 11q22-q23.1 and 11q25-qterm are frequent in sporadic breast but not colorectal cancers. Oncogene 14: 431-437.

Kroemer G & Reed JC (2000) Mitochondrial control of cell death. Nature Med 6: 513-519.

Kuramochi M, Fukuhara H, Nobukuni T, Kanbe T, Maruyama T, Ghosh HP, Pletcher M, Isomura M, Onizuka M, Kitamura T, Sekiya T, Reeves RH & Murakami Y (2001) TSLC1 is a tumor-suppressor gene in human non-small-cell lung cancer. Nature Genet 27: 427-430.

Kuukasjärvi T, Karhu R, Tanner M, Kähkönen M, Schäffer A, Nupponen N, Pennanen S, Kallioniemi A, Kallioniemi O-P & Isola J (1997) Genetic heterogeneity and clonal evolution underlying development of asynchronous metastasis in human breast cancer. Cancer Res 57: 1597-1604.

Körkkö J, Annunen S, Pihjalamaa T, Prockop DJ & Ala-Kokko L (1998) Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 95: 1681-1685.

Laake K, Jansen L, Hahnemann JM, Brøndum-Nielsen K, Lönnqvist T, Kääriäinen H, Sankila R, Lähdesmäki A, Hammarström L, Yuen J, Tretli S, Heiberg A, Olsen JH, Tucker M, Kleinerman R & Børresen-Dale A-L (2000a) Characterization of ATM mutations in 41 Nordic families with ataxia telangiectasia. Hum Mutat 16: 232-246.

Laake K, Launonen V, Niederacher D, Gudlaugsdottir S, Seitz S, Rio P, Champème M-H, Bièche I, Birnbaum D, White G, Sztan M, Sever N, Plummer S, Osorio A, Broeks A, Huusko P, Spurr N, Borg Å, Cleton-Jansen AM, van't Veer L, Benitez J, Casey G, Peterlin B, Olah E, Varley J, Bignon Y-J, Scherneck S, Sigurdardottir V, Lidereau R, Eyfjord J, Beckmann MW, Winqvist R, Skovlund E, Børresen-Dale A-L & the Breast Cancer Somatic Genetics Consortium (1999) Loss of heterozygosity at 11q23.1 and survival in breast cancer: results of a large European study. Genes Chrom Cancer 25: 212-221.

Laake K, Ødegard Å, Andersen TI, Bukholm IK, Kåresen R, Nesland JM, Ottestad L, Shiloh Y & Børresen-Dale A-L (1997) Loss of heterozygosity at 11q23.1 in breast carcinomas: indication for involvement of a gene distal and close to ATM. Genes Chrom Cancer 18: 175-180.

Laake K, Telatar M, Geitvik GA, Hansen RØ, Heiberg A, Andersen AM, Gatti R & Børresen-Dale A-L (1998) Identical mutation in 55% of the ATM alleles in 11 Norwegian AT families: evidence for a founder effect. Eur J Hum Genet 6: 235-244.

Laake K, Vu P, Andersen TI, Erikstein B, Kårensen R, Lønning PE, Skovlund E & Børresen-Dale A-L (2000b) Screening breast cancer patients for Norwegian ATM mutations. Br J Cancer 83: 1650-1653.

Larson GP, Zhang G, Ding S, Foldenauer K, Udar N, Gatti RA, Neuberg D, Lunetta KL, Ruckdeschel JC, Longmate J, Flanagan S & Krontiris TG (1998) An allelic variant at the ATM locus is implicated in breast cancer susceptibility. Genet Test 1: 165-170.

Launonen V, Laake K, Huusko P, Niederacher D, Beckman MW, Barkardottir RB, Geirsdottir EK, Gudmundsson J, Rio P, Bignon Y-J, Seitz S, Scherneck S, Bièche I, Champème M-H, Birnbaum D, White G, Varley J, Sztán M, Olah E, Osorio A, Benitez J, Spurr N, Velikonja N, Peterlin B, Borg Å, Cleton-Jansen A-M, Devilee P, Bloigu R, Lidereau R, Børresen-Dale A-L, Winqvist R & the Breast Cancer Somatic Genetics Consortium (1999) European multicenter study on LOH of APOC3 at 11q23 in 766 breast cancer patients: relation to clinical variables. Br J Cancer 80: 879-882.

Lee C-H & Chung JH (2001) The hCds1 (Chk2)-FHA domain is essential for a chain of phosphorylation events on hCds1 that is induced by ionizing radiation. J Biol Chem 276: 30537-30541.

Lee MS, Ogg S, Xu M, Parker LL, Donoghue DJ, Maller JL & Piwnica-Worms H (1992) cdc25+ encodes a protein phosphatase that dephosphorylates $p34^{cdc2}$. Mol Biol Cell 3: 73-84.

Lee SB, Kim SH, Bell DW, Wahrer DCR, Schiripo TA, Jorczak MM, Sgroi DC, Garber JE, Li FP, Nichols KE, Varley JM, Godwin AK, Shannon KM, Harlow E & Haber DA (2001) Destabilization of CHK2 by a missense mutation associated with Li-Fraumeni syndrome. Cancer Res 61: 8062-8067.

Lee W-H, Morton RA, Epstein JI, Brooks JD, Cambell PA, Bova GS, Hsieh W-S, Isaacs WB & Nelson WG (1994) Cytidine methylation of regulatory sequences near the π-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proc Natl Acad Sci USA 91: 11733-11737.

Le Page F, Kwoh EE, Avrutskaya A, Gentil A, Leadon SA, Sarasin A & Cooper PK (2000) Transcription-coupled repair of 8-oxoguanine: requirement for XPG, TFIIH, and CBS and implications for Cocayne syndrome. Cell 101: 159-171.

Levy-Lahad E, Lahad A, Eisenberg S, Dagan E, Paperna T, Kasinetz L, Catane R, Kaufman B, Beller U, Renbaum P & Gershoni-Baruch R (2001) A single nucleotide polymorphism in the RAD51 gene modifies cancer risk in BRCA2 but not BRCA1 carriers. Proc Natl Acad Sci USA 98: 3232-3236.

Li B, Goyal J, Dhar S, Dimri G, Evron E, Sukumar S, Wazer DE & Band V (2001) CpG methylation as a basis for breast tumor-specific loss of NES1/Kallikrein 10 espression. Cancer Res 61: 8014-8021.

Li FP & Fraumeni Jr JF (1969) Soft-tissue sarcomas, breast cancer, and other neoplasms: a familial syndrome? Ann Intern Med 71: 747-752.

Li FP, Fraumeni Jr JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA & Miller RW (1988) A cancer family syndrome in twenty-four kindreds. Cancer Res 48: 5358-5362.

Li H, Zhao L-L, Funder JW & Liu J-P (1997) Protein phosphatase 2A inhibits nuclear telomerase activity in human breast cancer cells. J Biol Chem 272: 16729-16732.

Lim D-S, Kim S-T, Xu B, Maser RS, Lin J, Petrini JHJ & Kastan MB (2000) ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. Nature 404: 613-617.

Lim D-S, Kirsch DG, Canman CE, Ahn J-H, Ziv Y, Newman LS, Darnell RB, Shiloh Y & Kastan MB (1998) ATM binds to β-adaptin in cytoplasmic vesicles. Proc Natl Acad Sci USA 95: 10146-10151.

Lindahl T & Wood RD (1999) Quality control by DNA repair. Science 286: 1897-1905.

Liotta LA, Goldfarb RH, Brundage R, Siegal GP, Terranova V & Garbisa S (1981) Effect of plasminogen activator (urokinase), plasmin and thrombin on glycoprotein and collagenous components of basement membrane. Cancer Res 41: 4629-4636.

Liu Q, Guntuku S, Cui X-S, Matsuoka S, Cortez D, Tamai K, Luo G, Carattini-Rivera S, DeMayo F, Bradley A, Donehower LA & Elledge SJ (2000) Chk1 is an essential kinase that is regulated by Atr and required for the G_2/M DNA damage checkpoint. Genes Dev 14: 1448-1459.

Liu Y & West SC (2002) Distinct functions of BRCA1 and BRCA2 in double-strand break repair. Breast Cancer Res 4: 9-13.

Lobaccaro J-M, Lumbroso S, Belon C, Galtier-Dereure F, Bringer J, Lesimple T, Heron J-F, Pujol H & Sultan C (1993) Male breast cancer and the androgen receptor gene. Nature Genet 5: 109-110.

Loeb LA (1991) Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 51: 3075-3079.

Lopez-Girona A, Tanaka K, Chen X-B, Baber BA, McGowan CH & Russel P (2001) Serine-345 is required for Rad3-dependent phosphorylation and function of checkpoint kinase Chk1 in fission yeast. Proc Natl Acad Sci USA 98: 11289-11294.

Lorincz MC & Groudine M (2001) $C^{m}C$(a/t)GG methylation: A new epigenetic mark in mammalian DNA? Proc Natl Acad Sci USA 98: 10034-10036.

Lorincz MC, Schübeler D, Goeke SC, Walters M, Groudine M & Martin DIK (2000) Dynamic analysis of proviral induction and de novo methylation: Implications for a histone deacetylase-independent, methylation density-dependent mechanism of transcriptional repression. Mol Cell Biol 20: 842-850.

Lu D & Yunis JJ (1992) Cloning, expression and localization of an RNA helicase gene from a human lymphoid cell line with chromosomal breakpoint 11q23.3. Nucleic Acid Res 20: 1967-1972.

Mailand N, Falck J, Lukas C, Syljuåsen RG, Welcker M, Bartek J & Lukas J (2000) Rapid destruction of Cdc25A in response to DNA damage. Science 288: 1425-1429.
Maillet P, Chappuis PO, Vaudan G, Dobbie Z, Müller H, Hutter P & Sappino A-P (2000) A polymorphism in the ATM gene modulates the penetrance of hereditary non-polyposis colorectal cancer. Int J Cancer 88: 928-931.
Malkin D, Li FP, Strong LC, Fraumeni Jr JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA & Friend SH (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250: 1233-1238.
Malone CS, Miner MD, Doerr JR, Jackson JP, Jacobsen SE, Wall R & Teitell M (2001) C^{m}C(A/T)GG DNA methylation in mature B cell lymphoma gene silencing. Proc Natl Acad Sci USA 98: 10404-10409.
Marfatia SM, Lue RA, Branton D & Chishti AH (1995) Identification of the protein 4.1 binding interface on glycophorin C and p55, a homologue of the Drosophila discs-large tumor suppressor protein. J Biol Chem 270: 715-719.
Marsischky GT, Lee S, Griffith J & Kolodner RD (1999) Saccharomyces cerevisiae MSH2/6 complex interacts with Holliday junctions and facilitates their cleavage by phage resolution enzymes. J Biol Chem 274: 7200-7206.
Matsuoka S, Huang M & Elledge SJ (1998) Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science 282: 1893-1897.
Matsuoka S, Rotman G, Ogawa A, Shiloh Y, Tamai K & Elledge SJ (2000) Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. Proc Natl Acad Sci USA 97: 10389-10394.
Maya R, Balass M, Kim S-T, Shkedy D, Leal J-F, Shifman O, Moas M, Buschmann T, Ronai Z, Shiloh Y, Kastan MB, Katzir E & Oren M (2001) ATM-dependent phosphorylation of Mdm2 on serine 395: role in p53 activation by DNA damage. Genes Dev 15: 1067-1077.
Melchionna R, Chen X-B, Blasina A & McGowan CH (2000) Threonine 68 is required for radiation-induced phosphorylation and activation of Cds1. Nature Cell Biol 2: 762-765.
Menoyo A, Alazzouzi H, Espín E, Armengol M, Yamamoto H & Schwartz, Jr. S (2001) Somatic mutations in the DNA damage-response genes ATR and CHK1 in sporadic stomach tumors with microsatellite instability. Cancer Res 61: 7727-7730.
Meyn MS (1999) Ataxia-telangiectasia, cancer and the pathobiology of the ATM gene. Clin Genet 55: 289-304.
Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A & Skolnick M (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71.
Miller CW, Ikezoe T, Krug U, Hofmann W-K, Tavor S, Vegesna V, Tsukasaki K, Takeuchi S & Koeffler HP (2002) Mutations of the CHK2 gene are found in some osteosarcomas, but are rare in breast, lung, and ovarian tumors. Genes Chrom Cancer 33: 17-21.
Miller DG (1980) On the nature of susceptibility to cancer. Cancer 46: 1307-1318.
Miyashita T & Reed JC (1995) Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 80: 293-299.
Monni O, Hyman E, Mousses S, Bärlund M, Kallioniemi A & Kallioniemi O-P (2001) From chromosomal alterations to target genes for therapy: integrating cytogenetic and functional genomic views of the breast cancer genome. Semin Cancer Biol 11: 395-401.
Müller M, Strand S, Hug H, Heinemann E-M, Walczak H, Hofmann WJ, Stremmel W, Krammer PH & Galle PR (1997) Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. J Clin Invest 99: 403-413.
Müller M, Wilder S, Bannasch D, Israeli D, Lehlbach K, Li-Weber M, Friedman SL, Galle PR, Stremmel W, Oren M & Krammer PH (1998) p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. J Exp Med 188: 2033-2045.

Mulligan LM, Kwok JBJ, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, Ponder MA, Telenius H, Tunnacliffe A & Ponder BAJ (1993) Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 363: 458-460.

Murakami Y, Nobukuni T, Tamura K, Maruyama T, Sekiya T, Arai Y, Gomyou H, Tanigami A, Ohki M, Cabin D, Frischmeyer P, Hunt P & Reeves RH (1998) Localization of tumor suppressor activity important in nonsmall cell lung carcinoma on chromosome 11q. Proc Natl Acad Sci USA 95: 8153-8158.

Nan X, Ng H-H, Johnson CA, Laherty CD, Turner BM, Eisenman RN & Bird A (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393: 386-389.

Nathanson KL & Weber BL (2001) ‘Other’ breast cancer susceptibility genes: searching for more holy grail. Hum Mol Genet 10: 715-720.

Nathanson KL, Wooster R & Weber B (2001) Breast cancer genetics: What we know and what we need. Nature Med 7: 552-556.

Negrini M, Rasio D, Hampton GM, Sabbioni S, Rattan S, Carter SL, Rosenberg AL, Schwartz GF, Shiloh Y, Cavenee WK & Croce CM (1995) Definition and refinement of chromosome 11 regions of loss of heterozygosity in breast cancer: identification of a new region at 11q23.3. Cancer Res 55: 3003-3007.

Negrini M, Sabbioni S, Possati L, Rattan S, Corallini A, Barbanti-Brodano G & Croce CM (1994) Suppression of tumorigenicity of breast cancer cells by microcell-mediated chromosome transfer: studies on chromosomes 6 and 11. Cancer Res 54: 1331-1336.

Nicoll G, Crichton DN, McDowell HE, Kernohan N, Hupp TR & Thompson AM (2001) Expression of the hypermethylated in cancer gene (HIC-1) is associated with good outcome in human breast cancer. Br J Cancer 85: 1878-1882.

NIH Consensus Development Conference (1980) Steroid receptors in breast cancer. Cancer 46: 2759-2963.

Nouspikel T, Lalle P, Leadon SA, Cooper PK & Clarkson SG (1997) A common mutational pattern in Cocayne syndrome patients from xeroderma pigmentosum group G: implications for a second XPG function. Proc Natl Acad Sci USA 94: 3116-3121.

O’Connell P, Pekkel V, Fuqua SAW, Osborne CK, Clark GM & Allred DC (1998) Analysis of loss of heterozygosity in 399 premalignant breast lesions at 15 genetic loci. J Natl Cancer Inst 90: 697-703.

Oda K, Arakawa H, Tanaka T, Matsuda K, Tanikawa C, Mori K, Nishimori H, Tamai K, Tokino T, Nakamura Y & Taya Y (2000) p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. Cell 102: 849-862.

Oka A & Takashima S (1998) Expression of the ataxia-telangiectasia gene (ATM) product in human cerebellar neurons during development. Neurosci Letters 252: 195-198.

Okano M, Bell DW, Haber DA & Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99: 247-257.

Olivier M & Hainaut P (2001) TP53 mutation patterns in breast cancers: searching for clues of environmental carcinogenesis. Semin Cancer Biol 11: 353-360.

Olsen JH, Hahnemann JM, Børresen-Dale A-L, Brøndum-Nielsen K, Hammarström L, Kleinerman R, Kääriäinen H, Lönnqvist T, Sankila R, Seersholm N, Tretli S, Yuen J, Boice, Jr. JD & Tucker M (2001) Cancer in patients with ataxia-telangiectasia and in their relatives in the Nordic countries. J Natl Cancer Inst 93: 121-127.

Ory K, Legros Y, Augin C & Soussi T (1994) Analysis of the most representative tumour-derived p53 mutants reveals that changes in protein conformation are not correlated with loss of transactivation or inhibition of cell proliferation. EMBO J 13: 3496-3504.

Oshimura M, Kugoh H, Koi M, Shimizu M, Yamada H, Satoh H & Barrett JC (1990) Transfer of a normal human chromosome 11 suppresses tumorigenicity of some but not all tumor cell lines. J Cell Biochem 42: 135-142.

Park M (1998) Oncogenes. In: Vogelstein B & Kinzler KW (eds) The genetic basis of human cancer: 205-228. McGraw-Hill, New York.

Parkin DM, Pisani P & Ferlay J (1993) Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer 54: 594-606.

Parsons R, Li G-M, Longley MJ, Fang W-H, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B & Modrich P (1993) Hypermutability and mismatch repair deficiency in RER+ tumor cells. Cell 75: 1227-1236.

Paull TT & Gellert M (1998) The 3' to 5' exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. Mol Cell 1: 969-979.

Peltomäki P (2001) Deficient DNA mismatch repair: a common etiologic factor for colon cancer. Hum Mol Genet 10: 735-740.

Peltonen L, Jalanko A & Varilo T (1999) Molecular genetics of the Finnish disease heritage. Hum Mol Genet 8: 1913-1923.

Peng C-Y, Graves PR, Thoma RS, Wu Z, Shaw AS & Piwnica-Worms H (1997) Mitotic and G2 checkpoint control: regulation of 14-3-3 protein by phosphorylation of Cdc25C on serine-216. Science 277: 1501-1505.

Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge Ø, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO & Botstein D (2000) Molecular portraits of human breast tumours. Nature 406: 747-752.

Petrini JHJ (2000) The Mre11 complex and ATM: collaborating to navigate S phase. Curr Opin Cell Biol 12: 293-296.

Petrini JHJ, Walsh ME, DiMare C, Chen C-N, Korenberg JR & Weaver DT (1995) Isolation and characterization of the human MRE11 homologue. Genomics 29: 80-86.

Pharoah PDP, Antoniou A, Bobrow M, Zimmern RL, Easton DF & Ponder BAJ (2002) Polygenic susceptibility to breast cancer and implications for prevention. Nature Genet 31: 33-36.

Phelan CM, Rebbeck TR, Weber BL, Devilee P, Ruttledge MH, Lynch HT, Lenoir GM, Stratton MR, Easton DF, Ponder BAJ, Cannon-Allbright L, Larsson C, Goldgar DE & Narod SA (1996) Ovarian cancer risk in BRCA1 carriers is modified by the HRAS1 variable number of tandem repeat (VNTR) locus. Nature Genet 12: 309-311.

Phillips KK, Welch DR, Miele ME, Lee J-H, Wei LL & Weissman BE (1996) Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. Cancer Res 56: 1222-1227.

Pippard EC, Hall AJ, Barker DJP & Bridges BA (1988) Cancer in homozygotes and heterozygotes of ataxia-telangiectasia and xeroderma pigmentosum in Britain. Cancer Res 48: 2929-2932.

Polyak K, Xia Y, Zweier JL, Kinzler KW & Vogelstein B (1997) A model for p53-induced apoptosis. Nature 389: 300-305.

Rahman N, Teare MD, Seal S, Renard H, Mangion J, Cour C, Thompson D, Shugart Y, Eccles D, Devilee P, Meijers H, Nathanson KL, Neuhausen SL, Weber B, Chang-Claude J, Easton DF, Goldgar D & Stratton M (2000) Absence of evidence for a familial breast cancer susceptibility gene at chromosome 8p12-p22. Oncogene 19: 4170-4173.

Rebbeck TR, Couch FJ, Kant J, Calzone K, DeShano M, Peng Y, Chen K, Garber JE & Weber BL (1996) Genetic heterogeneity in hereditary breast cancer: role of BRCA1 and BRCA2. Am J Hum Genet 59: 547-553.

Rebbeck TR, Kantoff PW, Krithivas K, Neuhausen S, Blackwood MA, Godwin AK, Daly MB, Narod SA, Garber JE, Lynch HT, Weber BL & Brown M (1999) Modification of BRCA1-associated breast cancer risk by the polymorphic androgen receptor CAG repeat. Am J Hum Genet 64: 1371-1377.

Rich T, Allen RL & Wyllie AH (2000) Defying death after DNA damage. Nature 407: 777-783.

Roberts SA, Spreadborough AR, Bulman B, Barber JBP, Evans DGR & Scott D (1999) Heritability of cellular radiosensitivity: a marker of low-penetrance predisposition genes in breast cancer? Am J Hum Genet 65: 784-794.

Robertson G, Coleman A & Lugo TG (1996) A malignant melanoma tumor suppressor on human chromosome 11. Cancer Res 56: 4487-4492.

Rodriguez C, Vallès H, Causse A, Johannsdottir V, Eliaou J-F & Theillet C (2002) Involvement of ATM missense variants and mutations in a series of unselected breast cancer cases. Genes Chrom Cancer 33: 141-149.

Ross JS & Fletcher JA (1999) The HER-2/neu oncogene: prognostic factor, predictive factor and target for therapy. Semin Cancer Biol 9: 125-138.

Roylance R, Gorman P, Harris W, Liebmann R, Barnes D, Hanby A & Sheer D (1999) Comparative genomic hybridization of breast tumors stratified by histological grade reveals new insights into the biological progression of breast cancer. Cancer Res 59: 1433-1436.

Ruvolo PP, Deng X, Ito T, Carr BK & May WS (1999) Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A. J Biol Chem 274: 20296-20300.

Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnica-Worms H & Elledge SJ (1997) Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 277: 1497-1501.

Santoro MF, Annand RR, Robertson MM, Pend Y-W, Brady MJ, Mankovich JA, Hackett MC, Ghayur T, Walter G, Wong WW & Giegel DA (1998) Regulation of protein phosphatase 2A activity by caspase-3 during apoptosis. J Biol Chem 273: 13119-13128.

Sasaki T, Tian H, Kukita Y, Inazuka M, Tahira T, Imai T, Yamauchi M, Saito T, Hori T, Hashimoto-Tamaoki T, Komatsu K, Nikaido O & Hayashi K (1998) ATM mutations in patients with ataxia telangiectasia screened by a hierarchical strategy. Hum Mutat 12: 186-195.

Sato T, Tanigami A, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G & Nakamura Y (1990) Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. Cancer Res 50: 7184-7189.

Satoh H, Lamb PW, Dong J-T, Everitt J, Boreiko C, Oshimura M & Barrett JC (1993) Suppression of tumorigenicity of A549 lung adenocarcinoma cells by human chromosomes 3 and 11 introduced via microcell-mediated chromosome transfer. Mol Carcinogen 7: 157-164.

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Patanjali SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NGJ, Taylor MR, Arlett CF, Miki T, Weissman SM, Lovett M, Collins FS & Shiloh Y (1995a) A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268: 1749-1753.

Savitsky K, Sfez S, Tagle DA, Ziv Y, Sartiel A, Collins FS, Shiloh Y & Rotman G (1995b) The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. Hum Mol Genet 4: 2025-2032.

Savitsky K, Ziv Y, Bar-Shira A, Gilad S, Tagle DA, Smith S, Uziel T, Sfez S, Nahmias J, Sartiel A, Eddy RL, Shows TB, Collins FS, Shiloh Y & Rotman G (1996) A human gene (DDX10) encoding a putative DEAD-box RNA helicase at 11q22-q23. Genomics 33: 199-206.

Schmidt L, Duh F-M, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, Allikmets R, Chidambaram A, Bergerheim UR, Feltis JT, Casadevall C, Zamarron A, Bernues M, Richard S, Lips CJ, Walther MM, Tsui LC, Geil L, Orcutt ML, Stackhouse T, Lipan J, Slife L, Brauch H, Decker J, Niehans G, Hughson MD, Moch H, Storkel S, Lerman MI, Linehan WM & Zbar B (1997) Germline and somatic muations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nature Genet 16: 68-73.

Schubert EL, Lee MK, Mefford HC, Argonza RH, Morrow JE, Hull J, Dann JL & King M-C (1997) BRCA2 in American families with four or more cases of breast or ovarian cancer: recurrent and novel mutations, variable expression, penetrance and the possibility of families whose cancer is not attributable to BRCA1 or BRCA2. Am J Hum Genet 60: 1031-1040.

Schwendel A, Richard F, Langreck H, Kaufmann O, Lage H, Winzer KJ, Petersen I & Dietel M (1998) Chromosome alterations in breast carcinomas: frequent involvement of DNA losses including chromosomes 4q and 21q. Br J Cancer 78: 806-811.

Scott D, Barber JBP, Spreadborough AR, Burrill W & Roberts SA (1999) Increased chromosomal radiosensitivity in breast cancer patients: a comparison of two assays. Int J Radiat Biol 75: 1-10.

Scott D, Spreadborough A, Levine E & Roberts SA (1994) Genetic predisposition in breast cancer. Lancet 344: 1444.

Scully R, Anderson SF, Chao DM, Wei W, Ye L, Young RA, Livingston DM & Parvin JD (1997) BRCA1 is a component of the RNA polymerase II holoenzyme. Proc Natl Acad Sci USA 94: 5605-5610.

Sebastian B, Kakizuka A & Hunter T (1993) Cdc25M2 activation of cyclin-dependent kinases by dephosphorylation of threonine-14 and tyrosine-15. Proc Natl Acad Sci USA 90: 3521-3524.

Seitz S, Rohde K, Bender E, Nothnagel A, Kölble K, Schlag PM & Scherneck S (1997) Strong indication for a breast cancer susceptibility gene on chromosome 8p12-p22: linkage analysis in German breast cancer families. Oncogene 14: 741-743.

Seizinger BR, Klinger HP, Junien C, Nakamura Y, Le Beau M, Cavenee W, Emanuel B, Ponder B, Naylor S, Mitelman F, Louis D, Menon A, Newsham I, Decker J, Kaebling M, Henry I & Deimling AV (1991) Report of the committee on chromosome and gene loss in human neoplasia. Cytogenet Cell Genet 58: 1080-1096.

Semba S, Ouyang H, Han S-Y, Kato Y & Horii A (2000) Analysis of the candidate target genes for mutation in microsatellite instability-positive cancers of the colorectum, stomach, and endometrium. Int J Oncol 16: 731-737.

Serova OM, Mazouyer S, Puget N, Dubois V, Tonin P, Shugart YY, Goldgar D, Narod SA, Lynch HT & Lenoir GM (1997) Mutations in BRCA1 and BRCA2 in breast cancer families: are there more breast cancer-susceptibility genes? Am J Hum Genet 60: 486-495.

Shafman T, Khanna KK, Kedar P, Spring K, Kozlov S, Yen T, Hobson K, Gatei M, Zhang N, Watters D, Egerton M, Shiloh Y, Kharbanda S, Kufe D & Lavin MF (1997) Interaction between ATM protein and c-Abl in response to DNA damage. Nature 387: 520-523.

Shafman TD, Levitz S, Nixon AJ, Gibans L-A, Nichols KE, Bell DW, Ishioka C, Isselbacher KJ, Gelman R, Garber J, Harris JR & Haber DA (2000) Prevalence of germline truncating mutations in ATM in women with a second breast cancer after radiation therapy for a contralateral tumor. Genes Chrom Cancer 27: 124-129.

Shak S, for the Herceptin Multinational Investigator Study Group (1999) Overview of the trastuzumab (Herceptin) anti-HER2 monoclonal antibody clinical program in HER2-overexpressing metastatic breast cancer. Semin Oncol 26(suppl 12): 71-77.

Shen C-Y, Yu J-C, Lo Y-L, Kuo C-H, Yue C-T, Jou Y-S, Huang C-S, Lung J-C & Wu C-W (2000) Genome-wide search for loss of heterozygosity using laser capture microdissected tissue of breast carcinoma: an implication for mutator phenotype and breast cancer pathogenesis. Cancer Res 60: 3884-3892.

Shibata A, Tsai YC, Press MF, Henderson BE, Jones PA & Ross RK (1996) Clonal analysis of bilateral breast cancer. Clin Cancer Res 2: 743-748.

Shibata D & Aaltonen LA (2001) Genetic predisposition and somatic diversification in tumor development and progression. Adv Cancer Res 80: 83-114.

Shieh S-Y, Ahn J, Tamai K, Taya Y & Prives C (2000) The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. Genes Dev 14: 289-300.

Shinohara A, Ogawa H, Matsuda Y, Ushio N, Ikeo K & Ogawa T (1993) Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA. Nature Genet 4: 239-243.

Shoemaker DD, Schadt EE, Armour CD, He YD, Garret-Engele P, McDonagh PD, Loerch PM, Leonardson A, Lum PY, Cavet G, Wu LF, Altschuler SJ, Edwards S, King J, Tsang JS, Schimmack G, Schelter JM, Koch J, Ziman M, Marton MJ, Li B, Cundiff P, Ward T, Castle J, Krolewski M, Meyer MR, Mao M, Burchard J, Kidd MJ, Dai H, Phillips JW, Linsley PS, Stoughton R, Scherer S & Boguski MS (2001) Experimental annotation of the human genome using microarray technology. Nature 409: 922-927.

Sidransky D, Tokino T, Helzlsouer K, Zhenbauer B, Rauch G, Shelton B, Prestigiacomo L, Vogelstein B & Davidson N (1992) Inherited p53 gene mutations in breast cancer. Cancer Res 52: 2984-2986.

Smilenov LB, Brenner DJ & Hall EJ (2001) Modest increased sensitivity to radiation oncogenesis in ATM heterozygous versus wild-type mammalian cells. Cancer Res 61: 5710-5713.

Smith GCM, d'Adda di Fagagna F, Lakin ND & Jackson ST (1999) Cleavage and inactivation of ATM during apoptosis. Mol Cell Biol 19: 6067-6084.

Sodha N, Houlston RS, Williams R, Yuille MA, Mangion J & Eeles RA (2002) A robust method for detecting CHK2/RAD53 mutations in genomic DNA. Hum Mutat 19: 173-177.

Sodha N, Williams R, Mangion J, Bullock SL, Yuille MR & Eeles RA (2000) Screening hCHK2 for mutations. Science 289: 359.

Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE & Børresen-Dale AL (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98: 10869-10874.

Soussi T & Béroud C (2001) Assessing TP53 status in human tumours to evaluate clinical outcome. Nature Rev Cancer 1: 233-240.

Srivastava S, Zou Z, Pirollo K, Blattner W & Chang EH (1990) Germ-line transmission of a mutated p53 gene in a cancer-prone family with LI-Fraumeni syndrome. Nature 348: 747-749.

Stancovic T, Kidd AMJ, Sutcliffe A, McGuire GM, Robinson P, Weber P, Bedenham T, Bradwell AR, Easton DF, Lennox GG, Haites N, Byrd PJ & Taylor AMR (1998) ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. Am J Hum Genet 62: 334-345.

Stewart GS, Maser RS, Stancovic T, Bressan DA, Kaplan MI, Jaspers NGJ, Raams A, Byrd PJ, Petrini JH & Taylor AMR (1999) The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. Cell 99: 577-587.

Strachan T & Reed AP (1999) Cancer genetics. In: Human Molecular Genetics, 2nd edition: 428-444. BIOS Scientific Publishers Ltd. Oxford, UK.

Su Y & Swift M (2001) Outcomes of adjuvant radiation therapy for breast cancer in women with ataxia-telangiectasia mutations. JAMA 286: 2233-2234.

Sugasawa K, Okamoto T, Shimizu Y, Masutani C, Iwai S & Hanaoka F (2001) A multistep damage recognition mechanism for global genomic nucleotide excision repair. Genes Dev 15: 507-521.

Sun Z, Hsiao J, Fay DS & Stern DF (1998) Rad53 FHA domain associated with phosphorylated Rad9 in the DNA damage checkpoint. Science 281: 272-274.

Svejstrup JQ (2002) Mechanisms of transcription-coupled DNA repair. Nat Rev Mol Cell Biol 3: 21-29.

Swift M, Morrell D, Massey RB & Chase CL (1991) Incidence of cancer in 161 families affected by ataxia-telangiectasia. N Engl J Med 325: 1831-1836.

Swift M, Reitnauer PJ, Morrell D & Chase CL (1987) Breast and other cancers in families with ataxia-telangiectasia. N Engl J Med 316: 1289-1294.

Takagi Y, Futamura M, Yamaguchi K, Aoki S, Takahashi T & Saji S (2000) Alterations of the PPP2R1B gene located at 11q23 in human colorectal cancers. Gut 47: 268-271.

Tan JC, Indelicato SR, Narula SK, Zavodny PJ & Chou C-C (1993) Characterization of interleukin-10 receptors on human and mouse cells. J Biol Chem 268: 21053-21059.

Tavor S, Takeuchi S, Tsukasaki K, Miller CW, Hofmann W-K, Ikezoe T, Said JW & Koeffler HP (2001) Analysis of the CHK2 gene in lyphoid malignancies. Leuk Lymphoma 42:517-520

Taylor WR & Stark GR (2001) Regulation of the G2/M transition by p53. Oncogene 20: 1803-1815.

Telatar M, Teraoka S, Wang Z, Chun HH, Liang T, Castellvi-Bel S, Udar N, Børresen-Dale A-L, Chessa L, Bernatowska-Matuszkiewicz E, Porras O, Watanabe M, Junker A, Concannon P & Gatti R (1998) Ataxia-telangiectasia: identification and detection of founder-effect mutations in the ATM gene in ethnic populations. Am J Hum Genet 62: 86-97.

Teraoka SN, Malone KE, Doody DR, Suter NM, Ostrander EA, Daling JR & Concannon P (2001) Increased frequency of ATM mutations in breast carcinoma patients with early onset disease and positive family history. Cancer 92: 479-487.

Thai TH, Du F, Tsan JT, Jin Y, Phung A, Spillman MA, Massa HF, Muller CY, Ashfaq R, Mathis JM, Miller DS, Trask BJ, Baer R & Bowcock AM (1998) Mutations in the BRCA1-associated RING domain (BARD1) gene in primary breast, ovarian and uterine cancers. Hum Mol Genet 7: 195-202.

Thompson D, Szabo CI, Mangion J, Oldenburg RA, Odefrey F, Seal S, Barfoot R, Kroeze-Jansema K, Teare D, Rahman N, Renard H, KConFab Consortium, Mann G, Hopper JL, Buys SS, Andrulis IL, Senie R, Daly MB, West D, Ostrander EA, Offit K, Peretz T, Osorio A, Benitez J, Nathanson KL, Sinilnikova OM, Olàh E, Bignon Y-J, Ruiz P, Badzioch MD, Vasen HFA, Futreal AP, Phelan CM, Narod SA, Lynch HT, Ponder BAJ, Eeles RA, Meijers-Heijboer H, Stoppa-Lyonnet D, Couch FJ, Eccles DM, Evans DG, Chang-Claude J, Lenoir G, Weber BL, Devilee P, Easton DF, Goldgar DE & Stratton MR (2002) Evaluation of linkage of breast cancer

to the putative BRCA3 locus on chromosome 13q21 in 128 multiple case families from the Breast Cancer Linkage Consortium. Proc Natl Acad Sci USA 99: 827-831.

Tibbets RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, Shieh S-Y, Taya Y, Prives C & Abraham RT (1999) A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev 13: 152-157.

Tirkkonen M, Tanner M, Karhu R, Kallioniemi A, Isola J & Kallioniemi O-P (1998) Molecular cytogenetics of primary breast cancer by CGH. Genes Chrom Cancer 21: 177-184.

Tornaletti S & Hanawalt PC (1999) Effect of DNA lesions on transcription elongation. Biochimie 81: 139-146.

Toyooka KO, Toyooka S, Virmani AK, Sathyanarayana UG, Euhus DM, Gilcrease M, Minna JD & Gazdar AF (2001) Loss of expression and aberrant methylation of the CDH13 (H-Cadherin) gene in breast and lung carcinomas. Cancer Res 61: 4556-4560.

Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB & Issa J-P (1999) CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci USA 96: 8681-8686.

Trujillo KM, Yuan S-SF, Lee EY-HP & Sung P (1998) Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. J Biol Chem 273: 21447-21450.

Tsou HC, Teng DH-F, Ping XL, Brancolini V, Davis T, Hu R, Xie XX, Gruener AC, Schrager CA, Christiano AM, Eng C, Steck P, Ott J, Tavtigian SV & Peacocke M (1997) The role of MMAC1 mutations in early-onset breast cancer: causative in association with Cowden syndrome and excluded in BRCA1-negative cases. Am J Hum Genet 61: 1036-1043.

Tsuda H & Hirohashi S (1995) Identification of multiple breast cancers of multicentric origin by histological observations and distribution of allele loss on chromosome 16q. Cancer Res 55: 3395-3398.

Uchiumi F, Ohta T & Tanuma S (1996) Replication factor C recognizes 5´-phosphate ends of telomeres. Biochem Biophys Res Commun 229: 310-315.

Uziel T, Savitsky K, Platzer M, Ziv Y, Helbitz T, Nehls M, Boehm T, Rosenthal A, Shiloh Y & Rotman G (1996) Genomic organization of the ATM gene. Genomics 33: 317-320.

Vahteristo P, Eerola H, Tamminen A, Blomqvist C & Nevanlinna H (2001a) A probability model for predicting BRCA1 and BRCA2 mutations in breast and breast-ovarian cancer families. Br J Cancer 84: 704-708.

Vahteristo P, Tamminen A, Karvinen P, Eerola H, Eklund C, Aaltonen LA, Blomqvist C, Aittomäki C & Nevanlinna H (2001b) p53, CHK2, and CHK1 genes in Finnish families with Li-Fraumeni syndrome: further evidence of CHK2 in inherited cancer predisposition. Cancer Res 61: 5718-5722.

van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AAM, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R & Friend SH (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415: 530-536.

Varley JM, McGown G, Thorncroft M, Santibanez-Koref MF, Kelsey AM, Tricker KJ, Evans DGR & Birch JM (1997) Germ-line mutations of TP53 in Li-Fraumeni families: an extended study of 39 families. Cancer Res 57: 3245-3252.

Varon R, Vissinga C, Plazer M, Cerosaletti KM, Chrzanowska KH, Saar K, Beckmann G, Seemanová E, Cooper PR, Nowak NJ, Stumm M, Weemaes CMR, Gatti RA, Wilson RK, Digweed M, Rosenthal A, Sperling K, Concannon P & Reis A (1998) Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. Cell 93: 467-476.

Vehmanen P, Friedman LS, Eerola H, McClure M, Ward B, Sarantaus L, Kainu T, Syrjäkoski K, Pyrhönen S, Kallioniemi O-P, Muhonen T, Luce M, Frank TS & Nevanlinna H (1997a) Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes. Hum Mol Genet 6: 2309-2315.

Vehmanen P, Friedman LS, Eerola H, Sarantaus L, Pyrhönen S, Ponder BAJ, Muhonen T & Nevanlinna H (1997b) A low proportion of BRCA2 mutations in Finnish breast cancer families. Am J Hum Genet 60: 1050-1058.

Vogelstein B & Kinzler KW (1993) The multistep nature of cancer. Trends Genet 9: 138-141.

Vogt Sionov R & Haupt Y (1999) The cellular response to p53: the decision between life and death. Oncogene 18: 6145-6157.
Vorechovský I, Luo L, Lindblom A, Negrini M, Webster ADB, Croce CM & Hammarström L (1996) ATM mutations in cancer families. Cancer Res 56: 4130-4133.
Wallrapp C, Hähnel S, Müller-Pillasch F, Burghardt B, Iwamura T, Ruthenbürger M, Lerch MM, Adler G & Gress TM (2000) A novel transmembrane serine protease (TMPRSS3) overexpressed in pancreatic cancer. Cancer Res 60: 2602-2606.
Wang SS, Esplin ED, Li, JL, Huang L, Gazdar A, Minna J & Evans GA (1998) Alterations of the PPP2R1B gene in human lung and colon cancer. Science 282: 284-287.
Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ & Qin J (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 14: 927-939.
Watters D, Khanna KK, Beamish H, Birrell G, Spring K, Kedar P, Gatei M, Stenzel D, Hobson K, Kozlov S, Zhang N, Farrell A, Ramsay J, Gatti R & Lavin M (1997) Cellular localisation of the ataxia-telangiectasia (ATM) gene product and discrimination between mutated and normal forms. Oncogene 14: 1911-1921.
Weil MM, Kittrel FS, Yu Y, McCarthy M, Zabriskie RC & Ullrich RL (2001) Radiation induces genomic instability and mammary ductal dysplasia in Atm heterozygous mice. Oncogene 20: 4409-4411.
Weinberg RA (1989) Oncogenes, antioncogenes, and the molecular basis of multistep carcinogenesis. Cancer Res 49: 3713-3721.
Weissman BE, Saxon PJ, Pasquale SR, Jones GR, Geiser AG & Stanbridge EJ (1987) Introduction of a normal human chromosome 11 into a Wilms' tumor cell line controls its tumorigenic expression. Science 236: 175-180.
Wera S & Hemmings BA (1995) Serine/threonine protein phosphatases. Biochem J 311: 17-29.
Wilson VL, Smith RA, Ma S & Cutler RG (1987) Genomic 5-methyldeoxycytidine decreases with age. J Biol Chem 262: 9948-9951.
Winqvist R, Hampton GM, Mannermaa A, Blanco G, Alavaikko M, Kiviniemi H, Taskinen PJ, Evans GA, Wright FA, Newsham I & Cavenee WK (1995) Loss of heterozygosity for chromosome 11 in primary human breast tumors is associated with poor survival after metastasis. Cancer Res 55: 2660-2664.
Wood RD (1997) Nucleotide excision repair in mammalian cells. J Biol Chem 272: 23465-23468.
Wooster R, Bignell G, Lancaster J, Swift S, Seal S Mangion J, Collins N, Gregory S, Gumbs C, Micklem G, Barfoot R, Hamoudi M, Patel S, Rice C, Biggs P, Hashim Y, Smith A, Connor F, Arason A, Gudmundsson J, Ficenec D, Kelsell D, Ford D, Tonin P, Bishop DT, Spurr NK, Ponder BAJ, Eeles R, Peto J, Devilee P, Cornelisse C, Lynch H, Narod S, Lenoir G, Egilsson V, Barkadottir RB, Easton DF, Bentley DR, Futreal PA, Ashworth A & Stratton MR (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378: 789-792.
Wooster R, Mangion J, Eeles R, Smith S, Dowsett M, Averill D, Barrett-Lee P, Easton DF, Ponder BAJ & Stratton MR (1992) A germline mutation in the androgen receptor gene in two brothers with breast cancer and Reifenstein syndrome. Nature Genet 2: 132-134.
Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang M-CW, Hwang L-Y, Bowcock AM & Baer R (1996) Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nature Genet 14: 430-440.
Wu X, Ranganathan V, Weisman DS, Heine WF, Ciccone DN, O'Neill TB, Crick KE, Pierce KA, Lane WS, Rathbun G, Livingston DM & Weaver DT (2000) ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response. Nature 405: 477-482.
Wu X, Webster SR & Chen J (2001) Characterization of tumor-associated Chk2 mutations. J Biol Chem 276: 2971-2974.
Xu XL, Wu LC, Du F, Davis A, Peyton M, Tomizawa Y, Maitra A, Tomlinson G, Gazdar AF, Weissman BE, Bowcock AM, Baer R & Minna JD (2001) Inactivation of human SRBC, located within the 11p15.5-p15.4 tumor suppressor region, in breast and lung cancers. Cancer Res 61: 7943-7949.

Yan PS, Chen C-M, Shi H, Rahmatpanah F, Wei SH, Caldwell CW & Huang TH-M (2001) Dissecting complex epigenetic alterations in breast cancer using CpG island microarrays. Cancer Res 61: 8375-8380.

Yang Q, Mori I, Shan L, Nakamura M, Nakamura Y, Utsunomiya H, Yoshimura G, Suzuma T, Tamaki T, Umemura T, Sakurai T & Kakudo K (2001) Biallelic inactivation of retinoic acid receptor β2 gene by epigenetic change in breast cancer. Am J Pathol 158: 299-303.

Yarden RI, Pardo-Reoyo S, Sgagias M, Cowan KH & Brody LC (2002) BRCA1 regulates the G2/M checkpoint by activating Chk1 kinase upon DNA damage. Nature Genet 30: 285-289.

Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY-HP & Qin J (2002) SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. Genes Dev 16: 571-582.

Yokochi T & Robertson KD (2002) Preferential methylation of unmethylated DNA by mammalian de novo DNA methyltransferase Dnmt3a. J Biol Chem 277: 11735-11745.

Zhang X, Moréra S, Bates PA, Whitehead PC, Coffer AI, Hainbucher K, Nash RA, Sternberg MJE, Lindahl T & Freemont PS (1998a) Structure of an XRCC1 BRCT domain: a new protein-protein interaction module. EMBO J 17: 6404-6411.

Zhang Y, Cai X, Schlegelberger B & Zheng S (1998b) Assignment of human putative tumor suppressor genes ST13 (alias SNC6) and ST14 (alias SNC19) to human chromosome bands 22q13 and 11q24→q25 by in situ hybridization. Cytogenet Cell Genet 83: 56-57.

Zhao H & Piwnica-Worms H (2001) ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. Mol Cell Biol 21: 4129-2139.

Zhao S, Weng Y-C, Yuan S-SF, Lin Y-T, Hsu H-C, Lin S-CJ, Gerbino E, Song M-H, Zdzienicka MZ, Gatti RA, Shay JW, Ziv Y, Shiloh Y & Lee EY-HP (2000) Functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products. Nature 405: 473-477.

Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, Hayward N & Dracopoli NC (1996) Germline mutations in the $p16^{ink4a}$ binding domain of CDK4 in familial melanoma. Nature Genet 12: 97-99.

Zöchbauer-Müller S, Fong KM, Maitra A, Lam S, Geradts J, Ashfaq R, Virmani AK, Milchgrub S, Gazdar AF & Minna JD (2001) 5'CpG island methylation of the FHIT gene is correlated with loss of gene expression in lung and breast cancer. Cancer Res 61: 3581-3585.