Molecular Genetics of Cancers (Part II): Familial Cancers

Pimpicha Patmasiriwat, Ph.D.

Department of Clinical Microscopy, Faculty of Medical Technology, Mahidol University (Salaya Main Campus), Salaya, Nakhorn Prathom 73170, Thailand.

Siriraj Med J 2008;60:295-300
E-journal: http://www.sirirajmedj.com

In all malignancies, both in adult and pediatric patients, sporadic cancers occur as the major form while the hereditary cancer counterparts appear to be minor. A number of familial cancer syndromes have been recognized for over a hundred years, although, the elucidation at the molecular level has occurred only recently. Retinoblastoma, a pediatric cancer of the retina, was first linked to *RB1* tumor suppressor gene in 1988, and after then the other nineteen familial cancer syndromes, mainly inherited in an autosomal dominant manner, have been associated to 30 genes. On an initiating germline mutation background of tumor suppressor (TS) genes in familial cancers, the development and progression of tumor requires additional genetic events that can abrogate function of the remaining wild-type allele of the genes that have already mutated in the germline (Knudson's hypothesis) as well as the accumulation of more events in other TS genes or oncogenes. Genetic heterogeneity has been reported in some syndromes. Li-Fraumeni Syndrome (LFS), which was linked to both *TP53* and *hCHK*, is an example. These several associated genes in a single syndrome are usually involved in similar functional pathways. In some cancer syndromes (such as Cowden Syndrome (CS) and also LFS), the susceptibile genes involved multiple tissues rather than specific sites, and therefore, these familial cancer syndromes are characterized by the appearance of multiple tumors. Furthermore, low penetrating loci or modifier genetic loci may also influence tumor development. They may be responsible for some differences in cancer occurrence seen within or between families of the same cancer syndrome. The identification of genes associated with familial cancer syndromes enable a "molecular diagnosis" which makes the clinical assessment complete and allows directed cancer surveillance for the individuals determined to be at risk.

Mostly, the genes associated with familial cancers function as tumor suppressors, except for several genes such as *RET, MET, CDK4 and NMYC* which are oncogenes involving in Multiple Endocrine Neoplasia type 2 (MEN2), Hereditary Papillary Renal Cell Carcinoma (HPRCC), Familial Malignant Melanoma (FMM), and familial neuroblastoma, respectively. TS genes can be further classified as "gatekeepers" or "caretakers",[1] based on their specific functions. The gatekeeper genes act as negative regulators of tumor progression. They regulate the tumor growth by controlling cell proliferation or promoting cell death. Examples of gatekeepers are *TP53*, *APC* and VHL genes, in which mutated, non-functioning equivalents can cause LFS, Familial Adenomatous Polyposis Coli (FAP) and von-Hippel Lindau Disease (VHL), respectively. The caretaker genes function to take care of the genome's integrity by maintaining the genetic stability of a cell. Functional loss of these genes indirectly promotes tumor growth via increasing mutation rate. The caretaker genes include those classified as DNA repairing genes. *BRCA1* and *BRCA2* function in DNA -double strand break repair and their functional loss is associated with hereditary breast and ovarian cancer. A group of DNA mismatch repair genes, such as *hMSH2, hMSH6, hMLH1, hPMS1 and hPMS2*, is known to be responsible for hereditary nonpolyposis colorectal cancer (HNPCC). While some caretaker genes are linked to the inherited autosomal dominant conditions (i.e., *BRCA1/2* and the above mismatch repair genes), the others can be linked to inherited autosomal recessive conditions such as Xeroderma Pigmentosum (XP).

Understanding Knudson's '2-Hit' Hypothesis

The hypothesis was conceived to describe the events of tumor development in retinoblastoma. This hypothesis was later expanded to all hereditary cancers.[2] In the development of familial tumors, the first 'hit' occurs in a germline in a cancer susceptibile gene, and the second 'hit' occurs somatically in the other allele of the same gene. Knudson's hypothesis explains not only familial tumors, but also the development of some sporadic tumors in which the '2 hits' occurs somatically, affecting both alleles of the gene. Identification of the regions presented as loss of heterozygosity (LOH) in tumors allows the isolation of susceptibile genes and identification of mutation in the retained allele. Knudson's model has been expanded recently to include the following concepts; haploinsufficiency (i.e. *PTEN*[3]), methylator phenotype,[4] allelic loss on the location of a germline mutation (i.e. *APC*[2]), and 'third hits' involving loss of either TS allele following the first 2-hits events (*APC*[5]).

Clinical Evidence for the Presence of Cancer Genes in Families

In the 1800's, Paul Broca of France described clinical observations of a family with regard to female breast cancer spanning three generations.[6] The observations are still useful today to ascertain that a family has an increased cancer susceptibility. The presence of a highly penetrating cancer gene in families may be a *de novo* - new mutation or have been present for generations. Six important points have been suggested as a guideline for the presence of familial cancer:

1. Tumor development in an individual at a much younger age than that normally observed in the population for the sporadic type of specific cancers, often < 30 years.
2. Clustering of the same cancer type in related individuals.
3. The presence of bilateral cancer in an individual unlikely to result from a metastasis tumor (i.e. bilateral breast cancer), or multiple cancer sites in one organ (i.e. colorectal carcinoma (CRC) developing from multiple polyps).
4. The presence of cancer in the sex that in not normally affected, i.e. male breast cancer, in conjunction with female breast cancer in relatives.
5. The presentation of multiple primary malignancies in a single individual, i.e. breast and thyroid cancer seen in CS.
6. Cancer associated with other conditions such as mental retardation or melanin pigmentation (i.e. Peutz-Jeghers Syndrome (PJS))

The Genes Involved in Familial Tumor Syndromes

Autosomal dominantly inherited familial tumor syndromes have been grouped as pediatric syndromes, breast and ovarian cancer syndromes, endocrine neoplasia syndromes, intestinal cancer syndromes, renal carcinoma syndromes skin carcinoma syndromes and neurofibromatosis. The predominant tumors associated with each syndrome and the involved genes with their functions or classification are summarized in Table 1. The clinical characteristics of each syndrome are described elsewhere and are not included in this review.

The Breast and Ovarian Cancer Syndrome

Breast cancer is the most common malignancy in western females with an incidence of about 1/10 of the women population. In Thailand, the country's incidence rate of breast cancer is 17.2 per 100,000 women and the highest incidence clusters are in Bangkok.[7] The annual report of tumor registry in 2005 at Siriraj Hospital confirmed that breast cancer is the top leading cancer, with a frequency of 13.7% of all cancer types (Siriraj Hospital Tumor registry, statistical report, 2005). The hereditary nature of breast cancer is observed in between 5-10% of all breast cancer. Therefore, a positive family history for this disease is one of the highest risk factors. The presence of 3 or more affected individuals is generally required for a family to be classified as having familial breast cancer. In our country, mutations of *BRCA1* and *BRCA2* within the genes' coding regions were observed in one-half of familial and early-onset types of breast and ovarian cancer cases screened for genetic testing, while the other half is *BRCA1/2* mutation negative (*BRCA1/2 neg*).[8] Additional currently identified genes could also be linked to familial breast cancer. Hereditary breast cancer may be present as follows : (1) as a sole malignancy, (2) in conjunction with other tumors including ovarian cancer, (3) in females or in males, (4) as a component tumor of multi-tumor syndromes including LFS, CS, PJS and Ataxia Telangiectasia (A-T).

BRCA1 - Associated Syndrome

The first breast cancer susceptibility locus was mapped to 17q21 in 1990 and the *BRCA1* gene was identified in 1994.[9] Persons with early onset breast cancer (pre-menupausal) and / or the presence of ovarian cancer in either themselves or family members are likely to carry the *BRCA1* mutation and are included in the BRCA1-linked breast cancer family (OMIM#113705). This *BRCA1* associated breast cancer phenotype is an aggressive tumour, has a high mitotic index and is found sometimes with a somatic mutation of TP53 and is often negative for an estrogen receptor. In the BRCA1 mutation carriers, the estimation for the risk of developing breast cancer by age 70 was greater than 80% and the risk of developing ovarian cancer by the same age was greater than 40%.[10] BRCA1 is one of the large genes, consisting 24 exons, 22 of which are coding. Germline mutations of this gene have been detected in individuals with familial breast/ ovarian cancer. The BRCA1 mutations are scattered along the entire regions of the gene, including point missense and nonsense mutations, intragenic deletions and insertions and splice site mutation. The majority of BRCA1 mutations are predicted to cause a truncated protein.[11] Genetic changes at 3′UTR of BRCA1 has recently been reported and is suggested to be involved in breast cancer in African- American and some Asian populations. A racial difference has been observed for the mutation of the BRCA1 gene. Some specific founder mutations such as 185delAG and 5382insC are frequently seen in the Ashkenazi Jewish population[12] and are hardly identified or not seen at all in some other populations. Allelic loss at 17q12-21 has also been observed in familial breast and ovarian cancers,[13] and the introduction of the wild-type *BRCA1* into breast cancer cell lines has shown to inhibit growth in vitro, giving additional evidence for the tumour suppressor function of this gene.[14]

BRCA1 protein and function

The protein is an acid nuclear phosphoprotein of 220 KDa, consisting of 1863 amino acids with a zinc finger motif near the N-terminus. The full length protein has been identified in the nucleus of epithelial cells while some splice variant is likely present in cytoplasm.[15] The other important domains include the RAD51 binding domain, BARD1 (*BRCA1-Associated Ring Domain Protein1*) binding domain, BRCT (BRCA1 C-terminal) domain and the granins homology domain. BRCA1 is a multi-functional molecule. Involvement of BRCA1 in various cellular functions has been reviewed.[16,17] BRCA1 functions as a caretaker, playing an essential role in the maintenance of genomic integrity during DNA damage repair.[18,19] It is involved in DNA double strand break repair with the physical interaction with RAD51, a molecule known to be implicated in DNA repair using a homologous recombination-dependent mechanism.[17,20] In the repairing process, BRCA1 protein functions as a sensor of DNA damage by

TABLE 1. Autosomal dominant hereditary cancer syndromes and their associated genes.a

Syndrome	Predominant Tumors	Gene	Chrom. Location	Function/ Classification
Retinoblastoma	Pediatric retinal tumor	*RB1*	13q14	Cell cycle regulation, apoptosis
Wilms Tumour	Pediatric kidney tumor	*WT1*	11p13	Transcriptional regulation
Breast/Ovarian	Breast cancer, ovarian cancer, male breast cancer, prostate cancer	*BRCA1* *BRCA2*	17q21 13q12.3	DNA repair DNA repair
Li-Fraumeni (LFS)	Breast cancer, soft tissue sarcoma, brain tumors, adenocortical tumor, leukemia	*TP53* *hCHK2*	17p13 22q12.1	Transcription factor, cell cycle regulation and apoptosis, checkpoint kinase
Cowden Syndrome(CS)	Breast cancer, thyroid tumors (follicular adenoma, follicular carcinoma, papillary carcinoma), endometrial carcinoma)	*PTEN (/MMAC1/TEP1)*	10q23.3	Protein tyrosine phosphatase
Familial Adenomatous Polyposis (FAP)	Adenomatous polyps of the colorectum (increase gastro-intestinal cancer risk), Papillary thyroid carcinoma	*APC*	5q21	Regulation of cell proliferation, migration and adhesion, cytoskeletal reorganization, chromosome stability
Juvenile Polyposis Coli	Multiple Juvenile polyps in the GI tract, Colorectal and gastrointestinal malignancy	*SMAD4 (DPC4)* *BMPR1A*	18q21.1 10q21-22	Cytoplasmic mediator in the TGF-β signaling pathway, serine threonine kinase type 1 receptor
HNPCC	Colorectal and endometrial adenocarcinoma	*hMSH2, hMSH6, hMLH1, hPMS1, hPMS2*	2p22-21, 2p16, 3p21, 2q31-33, 7p22	DNA mismatch repair
Peutz-Jeghers (PJS)	Cancer of GI tract, breast cancer, testicular cancer, Gynaecological malignancies	*STK11 (/LKB1)*	19p13.3	Serine threonine kinase
Multiple Endocrine Neoplasia Type 2 (MEN 2)	Medullary thyroid carcinoma, phaeochromocytoma, mucosal neuromas	*RET*	10q11.2	Transmembrane receptor tyrosine kinase
MEN 1	Primary hyperparathyroidism, pancreatic islet cell tumors, anterior pituitary tumors	*MEN1*	11q13	Mediator of JunD transcriptional activity; possible role in TGF-β signalling via Smad3
Hereditary Paraganglioma and Phaeochromocytoma	Paraganglioma, phaeochromocytoma	*SDHD, SDHC, SDHB*	11q23 1q21 1p36.1-p35	Possible in the regulation of oxygen sensing and signalling
von-Hippel Lindau Disease (VHL)	Renal cell carcinoma, retinal and central nervous system haemangioblastomas, phaeochromocytoma	*VHL*	3p25	Promote fibronectin matrix assembly, a component of a ubiquitin ligase complex targeting HIF-1 for degradation
Hereditary Papillary Renal Cell Carcinoma	Papillary renal cell carcinoma	*MET*	7q31	Transmembrane receptor tyrosine kinase
Tuberous Sclerosis	Multiple hamartomas, renal cell carcinoma, astrocytoma	*TSC1* *TSC2*	9q34 16p13	Maintenance of the cytoskeleton
Familial Melanoma	Cutaneous malignant melanoma, pancreatic cancer,	*CDKN2A* *CDK4*	9p21 12q14	Cell cycle regulation at the G1/S
Nevoid BCC	Basal cell carcinoma	*PTC*	9q22.3	Negative regulator of cell division
NF1	Neurofibrosarcoma, astrocytomas, phaeochromocytoma, melanoma, rhabdomyosarcoma and chronic myeloid leukemia,	*NF1*	17q11	Negative regulator of RAS-mediated cellular proliferation
NF2	Bilateral vestibular schwannomas, meningiomas, spinal tumors, skin tumors	*NF2*	22q12	Maintenance of the cytoskeleton and adhesion, suppressor of cell motility and spreading
Carney Complex	Pituitary adenoma, testicular tumors, thyroid adenoma & carcinoma, breast ductal adenoma	PRKAR1A	17q22-24	Roles in cAMP pathway

[a]The data in this Table was modified from Marsh DJ, Zori RT. Genetic insights into familial cancers-update and recent discoveries. Oncoserve online 2004, Elsevier.

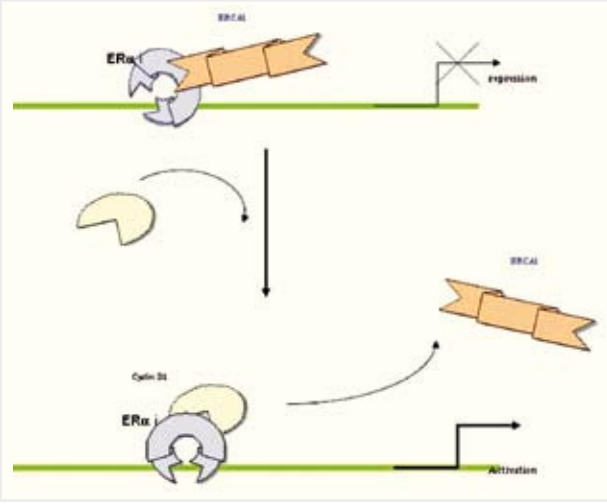

Fig 1. The proposed model in which cyclin D1 antagonizes BRCA1 repression of ERα activity via competing with BRCA1 binding to ERα (Modified from Wang et al, 2005).[39]

interacting with, as well as recruiting component molecules of the repairing 'supercomplex'. It interacts with participating proteins in the process, including RAD50, Mre11-NBS1, MSH2, MSH6 and proliferating cell nuclear antigen (PCNA).[18,21] The other main examples of BRCA1 functions are: (1) transcriptional regulation, as RNA polymerase II holoenzyme-bound protein;[22] (2) mitotic centrosome function, as a centrosome associated protein;[23] (3) cell cycle negative regulator through interacting with E2F and cyclins/CDKs;[24] and (4) cellular progression and genome stability, as a physiologic phosphorylation substrate of CDK2-cyclin complex.[25]

As mentioned above, breast/ovarian cancer patients carrying *BRCA1* mutations are often ER negative[26-28] and the mutation within BRCA1 gene is believed to be responsible for the breast /ovarian tumorigenesis in these carriers. However, the majority of breast /ovarian cancers are sporadic and often ER positive with the wild-type (intact sequence) BRCA1 and BRCA2. Cumulative evidences suggest the functional association between BRCA1 and ER. Inactivation of BRCA1 by antisense or gene deletion results in an obvious activation of ER (estrogen-independent).[29] Some functional defects of BRCA1 exist in sporadic breast cancer and are ER positive. Expression of the wt BRCA1 was absent or reduced in around 30% to 40% of ER positive sporadic breast cancers,[30-32] and loss of one allele of *BRCA1* was seen in 46% of the sporadic breast cancers.[33] These findings draw attention that functionally defective BRCA1 may also be related to the occurence of sporadic cancer, and the possibility of BRCA1 to regulate ER function has been suggested. CyclinD1 has been suggested to be the third-party molecule involved in the regulation of ER by BRCA1.

In the view of cancer cell biology, two classes of cancer genes exist. First, the genes that control genomic stability which include antimutators and DNA repair proteins. Second, the genes that control cell cycle and their functions involved in cellular proliferation and tumor growth.[34] *Cyclin D1* gene encodes the regulatory subunit of a holoenzyme (cyclinD1/CDK4) that phosphorylate and inactivate retinoblastoma tumour suppressor protein (pRb). The expression of *Cyclin D1* gene is controlled by oncogenic and mitotic signals. *CyclinD1* exerts a biological role in breast tumorigenesis and is thought to be a candidate mammary oncogene. Cyclin D1 is overexpressed in 30%-40% of ER positive human breast cancers and is associated with poor prognosis.[35,36] In transgenic mice, mammary gland-targeted cyclinD1 overexprssion could induce a mammary tumour.[37] In addition, *cyclin D1* -deleted mice were resistant to mammary tumourigenesis induced by Ras or ErbB2.[38]

Since BRCA1 is involved in various cellular mechanisms and functions to suppress tumour growth, the function of this protein might be interfered with during the progression of sporadic breast cancer. Upon the knowledge that BRCA1 function is reduced either by mutations (as seen in familial cancer) or decreased expression (as in sporadic cancer), and that *cyclin D1* is a candidate mammary oncogene, the association between BRCA1 and cyclin D1 has been of much interests. BRCA1 can repress ERα–dependent gene expression by binding with and so suppressing the ER α induced gene expression. Scientists could recently sho w that cyclin D1 antagonizes BRCA1 repression of estrogen responsive genes at an estrogen responsive element (ERE).[39] Cyclin D1 is capable of binding ERαat the same region of ERα as BRCA1 (Fig 1). There fore, it competes with BRCA1 for ERα binding and can rescue the ERα transcriptional repression by BRCA1. This antagonism by cyclin D1, if overcomes the BRCA1 suppression of its target genes, might be an important mechanism of breast cancinogenesis involved in both classes of cancer promoting genes.

BRCA2-Associated Syndrome

BRCA2 was identified in 1995[40] after being localized to chromosome 13q12-13 in 1994.[41] Germline mutation as well as loss of the wild-type allele has been reported in breast cancer families, suggesting a tumor suppressor function of this gene.[42-43] Families linked to this gene had a high risk of developing breast cancer, but did not have as high a risk of developing ovarian cancer as those *BRCA1*-linked families. Males in BRCA2 linked breast cancer families (OMIM# 600185) have an increased risk of developing male breast cancer and prostate cancer. Other BRCA2 associated cancers include pancreatic cancer, malignant melanoma, colon cancer and ovarian cancer (to a lesser extent than BRCA1 associated families).

The gene has 27 exons, with large exon 10 and 11, encoding a 390kDa nuclear protein.[15] The risk of *BRCA2* mutation carriers in developing breast cancer is approximately 84% by age 70.[44] BRCA2 protein contains 8 copies of 30-80 amino acid repeats (BCR) encoded by exon 11. This region is required for binding to RAD51, and BRCA2 has been shown to be involved in the dsDNA damage response pathway, in cooperating with BRCA1 and RAD51.[45]

Li- Fraumeni Syndrome

LFS (OMIM#151623) had been defined by observing familial clustering of certain types of malignancies including early onset breast cancer, soft tissue sarcomas, brain tumours, adenocortical tumours and leukemias.[46,47] The two molecular determinants for LFS are *TP53* and *hCHK2* (checkpoint kinase 2). *TP53* gene is commonly mutated in sporadic cancers and germline mutations of this gene have been identified in the patients with

LFS.[48,49] *TP53* is located on 17p13 and has 11 exons encoding a 53kDa nuclear phosphoprotein. Highly penetrant germline mutations associated with LFS have been reported throughout the gene, with the majority between exon 5 and 8.[50] The protein product (P53) contains conserved regions including SV40 large tumour antigen binding sites, nuclear localization signal and several phosphorylation sites, all necessary for its normal function.[47] P53 is a transcription factor which regulate genes that induce cell cycle arrest or apoptosis, especially when the cells are exposed to DNA damaging agents. It mediates arrest in the G1 phase[51] and may be also at the G2.[52,53] When the cell stops its cycle, the DNA is then allowed to be fixed by the repairing protein complex at the damaged sites. The transcriptional targets of p53 include p21, mdm-2, BAX and GADD45.[47]

hCHK2 is another susceptibile gene for LFS.[54] The CHK2 protein is a kinase which is phosphorylated by ATM in response to DNA damage (173,174[55,56]), and the CHK2 protein directly phosphorylates p53, indicating its role in p53 regulation after DNA damage.[57,58] Mutations within *hCHK2* gene have been reported and shown to result in either loss of ability to be phosphorylated by ATM- dependent phosphorylation or loss of CHK2 kinase activity, which leads to an inability of the cell to respond to DNA damage.[59]

CONCLUSION

Understanding of the genetic basis of familial cancers has led to the molecular diagnosis for many of these syndromes, and has allowed directed cancer surveillance for members of families at risk of developing the disease. Efficient mutation screening of some genes such as BRCA1, BRCA2, NF1 and NF2 is still challenging in many population groups due to the large size of these genes and the lack of mutation 'hot-spots'. However, for the small genes such as VHL, or a gene such as as RET where mutations are usually seen at specific sites, the genetic testing has been effectively incorporated into the clinical management of the affected families. Cumulative knowledge of the roles of cancer – associated genes in normal development, adult tissue and malignancy, as well as the observation of the additional 'hits' that occur in many cases in the cancer process, will be useful for the advanced improvement of cancer treatment by allowing the therapeutic intervention at a molecular level.

ACKNOWLEDGMENTS

A part of the content in this article was presented at the **Thailand Research Fund (TRF)-Senior Research Scholar Academic Conference 2008 – *'Human Molecular Genetics Conference: Molecular Genetics of Com-plex and Common Genetic Diseases'***, September 29, 2008, Royal River Hotel, Bangkok, Thailand.

REFERENCES

1. Kinzler KW, Volgenstein B.Landscaping the cancer terrain. Science 1998;-280:1036-7.
2. Tomlinson IPM, Roylance R, Houlston RS. Two hits revisted again. J Med Genet 2001;38:81-85.
3. Cook WD, McCaw BJ. Accommodating haploinsufficient tumour suppressor genes in Knudson's model. Oncogene 2000;19:3434-8.
4. Costello JF, Plass C. Methylation matters. J Med Genet 2001;38:285-303.
5. Lamlum H, Ilyas M, Rowan A, Clark S, Johnson V, Bell J, et al. The type of somatic mutation at APC in familial adenomatous polyposis is determined by the site of the germline mutation: a new facet to knudson's 'two-hit' hypothesis. Nature Medicine 1999;5:1071-5.
6. Schneider NR, Williams WR, Chaganti RSK. Genetic epidemiology of familial aggregation of cancer. Adv Cancer Res 1986;47:1-29.
7. Martin N, Cheirsilpa A. Breast. In: Sriplung H, Sontipong S, Martin N, Wiangnon S, Vootiprux V, Cheirsilpa A, Kanchanabat C, Khuhaptrma T, eds. Cancer in Thailand, Vol III, 1995-1997. Bangkok, Bangkok Medical Publisher, 2003:47-48.
8. Patmasiriwat P, Bhothisuwan K, Sinilnikova OM, Chopin S, Methakijvaroon S, Badzioch M, et al. Analysis of breast cancer susceptibility genes *BRCA1* and *BRCA2* in Thai familial and isolated early- onset breast and ovarian cancer. Human Mutat 2002;20:230.(Online,http://www.interscience.wiley.com/humanmutation/pdf/mutation/520.pdf).
9. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science 1994;266:66-71.
10. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risk of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994;343:692-5.
11. Shuttuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, Couch F, et al. A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene. Implications for presymtomatic testing and screening. JAMA 1995;5:535-41.
12. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Askenazi Jews. N Engl J Med 1997:336:1401-8.
13. Smith SA, Easton DF, Evans DG, Ponder BA. Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome. Nat Genet 1992;2:128-31.
14. Holt JT, Thompson ME, Szabo C, Robinson-Benion C, Arteaga CL, King MC, et al. Growth retardation and tumour inhibition by BRCA1. Nat Genet 1996;12:298-302.
15. Arver B, Du Q, Chen J, Luo L, Lindblom A. Hereditary breast cancer: a review. Sem Can Biol 2000;10:271-88.
16. Chen Y, Lee W, Chew HK. Emerging roles of BRCA1 in transcriptional regulation and DNA repair. J Cell Physiol 1999;181:385-92.
17. Scully R, Livingston DM. In search of the tumour–suppressor functions of BRCA1 and BRCA2. Nature 2000;408:429-32.
18. Wang Y, Cortez D,Yazdi P, Neff N, Elledge SI, Qin J. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 2000;14:927-39.
19. Cantor SB, Bell DW, Ganesan S, et al. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell 2001;105:149-60.
20. Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, et al. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 1997;88: 265-75.
21. Zhong Q, Chen CE, Li S, et al. Association of BRCA1 with the Rad50-Mre11-p95 complex and the DNA change response. Science 1999;285: 747-50.
22. Scully R, Anderson SF, Chao DM, Wei W, Ye L, Young RA, et al. BRCA1 is a component of the RNA polymerase II holoenzyme. Proc Natl Acad Sci USA 1997;94:5605-10.
23. Hsu LC, White RL. BRCA1 is associated with the centrosome during mitosis. Proc Natl Acad Sci USA 1998;95:12983-8.
24. Wang H, Shao N, Ding QM, Cui J, Reddy ES, Rao VN. BRCA1 proteins are transported to the nucleus in the absence of serum and splice variants BRCA1a, BRCA1b are tyrosine phosphoproteins that associate with E2F, cyclins and cyclin dependent kinase. Oncogene 1997;15:143-57.
25. Ruffner H, Jiang W, Craig AG, Hunter T, Verma IM. BRCA1 is phosphorylated at serine 1497 in vivo at a cyclin-dependent kinase 2 phosphorylation site. Mol Cell Biol 1999;19:4843-54.
26. Osin PP, Lakhani SR. The pathology of familial breast cancer: immunohistochemistry and molecular analysis. Breast Cancer Res 1999;1:36-40.
27. Hedenfalk I, Duggan D, Chen Y, et al. Gene expression profiles in hereditary breast cancer. N Engl J Med 2001;344:538-48.
28. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene expression signature as a predictor of survival in breast cancer. N Engl J Med 2002; 347:1999-2009.
29. Zheng L, Annah LA, Afshari CA, Lee WH, Boyer TG. BRCA1 mediates ligand-independent transcriptional repression of the estrogen receptor. Proc Natl Acad Sci USA 2001;98:9587-92.
30. Taylor J, Lymboura M, Pace PE, et al. An important role of BRCA1 in breast cancer progression is indicated by its loss in a large proportion of non-familial breast cancers. Int J Cancer 1998;79:334-42.
31. Wilson CA,Ramos L, Villasenor MR, et al. Localization of human BRCA1 and its loss in high grade, non-inherited breast carcinomas. Nat Genet 1999;21:236-40.
32. Lee WY, Jin YT, Chang TW, Lin PW, Su IJ. Immunolocalization of BRCA1 protein in normal breast tissue and sporadic invasive ductal carcinomas: a correlation with other biological parameters. Histopathology 1999;34:106-12.
33. Foulkes WD, Stefansson IM, Chappuis PO, et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl cancer Inst 2003;95:1482-5.

34. Kinsler KW, Volgenstein B. Cancer susceptibility genes. Gatekeepers and caretakers. Nature 1997;386:761-3.
35. Kenny FS, Hui R, Musgrove EA, et al. Overexpression of cyclin D1 messenger RNA predicts for poor prognosis in estrogen receptor-positive breast cancer. Clin Cancer Res 1999;5:2069-76.
36. Pestell RG, Albanese C, Reutens AT, Segall JE, Lee RJ, Arnold A. The cyclins and cyclin-dependent kinase inhibitors in hormonal regulation of proliferation and differentiation. Endocr Rev 1999;20:501-34.
37. Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV. Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature 1994;369:669-71.
38. Yu Q, Geng Y, Sicinsky P. Specific protection against breast cancer by cyclin D1 ablation. Nature 2001;411:1017-21.
39. Wang C, Fan S, Li Z, Fu M, Rao M, Ma Y, et al. Cyclin D1 antagonizes BRCA1 repression of estrogen receptor α activity. Cancer res 2005;65:6557-67.
40. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995;378:789-92.
41. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, et al. Localization of a breastcancer susceptibility gene BRCA2, to chromosome 13q12-13. Science 1994;265:2088-90.
42. Tavtigian SV, Simard J, Rommens J, Couch F, Shuttuck-Eidens D, Neuhausen S, et al. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. Nat Genet 1996;12:333-7.
43. Collins N, McManus R, Wooster R, Mangion J, Seal S, Lakhani SR, et al. Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13. Oncogene 1995;10:1673-5.
44. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, et al. Breast Cancer Linkage Consortium, Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 1998;62:676-89.
45. Chen JJ, Silver D, Cantor S, Livingston DM, Scully R. BRCA1, BRCA2 and RAD51 operate in a common response pathway. Cancer Res 1999; 59:1752S-6S.
46. Li FP, Fraumeni JF. Soft tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome. Ann Intern Med 1969;71:747-52.
47. Malkin D. The Li-Fraumeni Syndrome. In: Volgelstein B, Kinzler KW, eds. The genetic basis of human cancer.McGraw Hill Companies, New York, 1998:393-407.
48. Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, et al. Germline p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233-8.
49. Srivastava S, Zou ZQ, Pirollo K, Blattner W, Chang EH. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni Syndrome. Nature 1990;348:747-9.
50. Frebourg T, Barbier N, Yan YX, Garber JE, Dreyfus M, Fraumeni J, et al. Germ-line p53 mutations in 15 families with Li-Fraumeni Syndrome. Am J Hum Genet 1995;56:608-15.
51. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 1992;89:7491-5.
52. Mercer WE, Shields MT, Lin D, Appella E, Ullrich SJ. Growth suppression induced by wild-type p53 protein is accompanied by selective down-regulation of proliferating −cell nuclear antigen expression. Proc Natl Acad Sci USA 1991;88:1958-62.
53. Guillouf C, Rosselli F, Krishnaraju K, Moustacchi E, Hoffman B, Liebermann DA. P53 involvement in control of G2 exit of the cell cycle: role in DNA damage-induced apoptosis. Oncogene 1995;10:2263-70.
54. Bell DW, Varley JM, Szydio TE, Kang DH, Wahrer DCR, Shannon KE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni Syndrome. Science 1999;286:2528-31.
55. Matsuoka S, Huang MX, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science 1998;282:1893-7.
56. Chaturvedi P, Eng WK, Zhu Y, Mattern MR, Mishra R, Hurle MR, et al. Mammarian Chk2 is a downstream effectors of the ATM-dependent DNA damage checkpoint pathway. Oncogene 1999;18:4047-54.
57. Shieh S-Y, Ahn J, Tamai K, Taya Y, Prives C. The human homologs of checkpoint kinase Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. Genes Dev 2000;14:289-300.
58. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, et al. DNA damage- induced activation of p53 by the checkpoint kinase Chk2. Science 2000;287:1824-7.
59. Wu X, Webster SR, Chen J. Characterization of tumor-associated Chk2 mutations. J Biol Chem 2001;276:2971-4.