An overview of cancer genetics with focus on involvement of BRCA1/2 genes in breast carcinomas

Durr-e-Samin Tahir, Muhammad Saifur Rehman, Muhammad Shahnawaz ul Rehman

Abstract

Cancer is a kind of disease which can be explained at the molecular level as triggered by accretion of damaged deoxyribonucleic acid. There are several genes involved in cancer, mainly belonging to two classes called tumour suppressors and oncogenes. Other than the well-known breast cancer susceptibility 1/2 genes, there are several other genes involved in the development of breast and ovarian cancers. However, since the past two decades the focus of research has been on and breast cancer susceptibility 1/2 genes. The current review was planned to delve into the structure and function of breast cancer susceptibility 1/2 genes to augment research on the genetics of breast cancer. The understanding of tumour suppressor genes is also helpful in the analysis of mutational spectra and to determine the treatment strategies in clinical interventional studies.

Keywords: Oncogenes, Tumour suppressor genes, BRCA1, BRCA2.

Introduction

At the molecular level, cancer is characterised by the accumulation of damaged and unrepaired deoxyribonucleic acid (DNA). In the human body, there are around 50,000 suspected endogenous mediators which are considered to be the cause of DNA damage per cell per day.[1] Moreover, there are hundreds of exogenous agents, like cancer-causing chemicals and various harmful radiations which are responsible for damage to DNA.[2] Due to the mechanism provided by nature, living things stay within sustainable level of DNA damage without any severe level of morbidity. In case of defect of DNA repair process due to hereditary, epigenetic or somatic alterations, the result is the accumulation of un-repaired DNA which is the actual cause of cancer.[3] The transformation of normal cell to cancerous cell requires a series of several mutations in specific classes of genes.[4] Genes that are involved in cell proliferation, differentiation and death must mutate to transform a normal cell into a malignant cell. Genetic alterations are likely to occur at any stage of life or at any level of development; variations could fluctuate from whole chromosome to only a single nucleotide. Similarly, epigenetic changes result in activating or silencing of microribonucleic acid (RNA) which in turn is responsible for the expression of hundreds of genes.[5]

Major classes of genes contributing to carcinomas

In the study of cancer genetics over several decades, the concept of Mendelian recessive and dominant was integrated. Biologists working in the field of cancer genetics identified two major classes of genes involved in this disease known as proto-oncogenes (POGs) and tumour suppressor genes (TSGs).[6]

Proto-Oncogenes

POGs are normal genes present in every human male, female and many other organisms. It is involved in normal cell growth and proliferation,but can mutate to oncogenes. Since these are dominant genes, therefore it means that the activation of only one allele is enough for normal cellular growth.[7] Cancerous cells form due to mutation or over-expression of these genes. In most of the cases, morbidity related to oncogenes is caused by mutation in some other genes such as TSGs or environmental factors, like viral infection.

Tumour Suppressor Genes (TSGs)

TSGs are further divided into caretaker TSGs and gatekeeper TSGs.[7] A third class of TSGs is called landscape genes,the mutated form of which has been found to promote angiogenesis.[8]

These genes affect the functions of cell, such as genomic stability, DNA repair, senescence, inter-cellular communication, interactions of cell with matrix mortality and angiogenesis.[9] In contrast to POGs, caretaker and gatekeeper TSGs act in a recessive manner. Theoretically speaking, at the cellular level, loss of function or inactivation of both alleles is required for pathogenicity, but actually for some TSGs, like breast cancer susceptibility genes (BRCA)1 and 2, there is evidence of haplo-insufficiency.[10] Thus, only one mutated gene is enough for the onset and progression

University of Agriculture, Faisalabad, Pakistan.
Correspondence: Durr-e-Samin Tahir. Email: durre.samin@njc.edu.pk

of the disease.Normal functioning of gatekeeper TSGs is related to the direct prevention of growth of tumours by apoptosis, differentiation and suppression of proliferation, whereas caretaker TSGs indirectly suppress tumorigenesis and neoplastic development.[11] However, this classification of TSGs somewhere has become arbitrary because some genes like tumour suppressor gene 53 (TP53) and BRCA2 have exhibited functions of caretaker as well as gatekeeper genes.[12] Recent research is focussing on synergy of functional and regulatory role of oncogenes and TSGs mediated by micro-RNA.[13] Meta-analyses and research about epigenetic methylation of promoters of TSGs also play a vital role in the development of cancer treatment techniques.[14]

Involvement of different TSGs in breast cancer (BC)

The most widespread genetic cancers found globally are breast, ovarian and prostate cancers caused by TSGs.[15] There are several somatic and germline mutations involved in the process of tumorigenesis.[16] More widely explored high penetrance genes are TP53 and BRCA1/2.[17,18] TP53 is known to express in 20-35% of breast tumours for which almost 1400 variants have been described to be involved in Li-Fraumeni cancer syndrome.[19] The cadherin 1 (CDH1) gene was found to be involved in lobular type of breast tumours in BC.[20] The carrier women of CDH1 exhibit an elevated risk of 50% of having BC in their lifetime.[21] However,the prognosis in under-expression of CDH1 was found to be poorly associated and greater rate of metastasis was revealed in oestrogen receptor (ER)-positive BC patients.[22] The carriers of phosphatases protein (PTEN) gene mutation have 4% more chance of breast carcinoma involving clathrin signalling.[23] Mutant form of this gene is involved in triple negative and ductal carcinomas. The somatic mutation caused by liver kinase B1 (LKB1), a serein threonine kinase 11 (STK11)-interacting protein, which is directly responsible for Peutz-Jeghers syndrome (PJS) and mutation in this locus is accompanied by around 5 times more risk of BC in PJS patients.[22] LKB1 and PTEN genes are responsible for the carcinoma of squamous cell in lungs.[24] There is another TSG present on chromosome 22 at q21.1 called checkpoint kinase 2 (CHK2) that contains 14 exons where the gene product consisting of 543 amino acids with three important conserved functional domains which are: sickle cell disease (SCD)-regulatory domain on N-terminus rich in threonine, serine and glutamine, forkhead-associated (FHA) domain which is protein-to-protein interaction, and kinase domain on C terminus.[25] The CHK2 gene plays a significant role to respond the cell on double stranded breaks (DSBs). The mode of action of the gene is to act on all checkpoints of the cell cycle, and to accordingly regulate it

by DNA repair or apoptosis.[26] There are evidences of involvement of some other genes like partner and localiser of BRCA2 (PALB2, RAD51 Recombinase and BRCA1 interacting protein (BRIP1) in hereditary BC, because of interconnection of these genes with BRCA1/2 functioning.[27] The ataxia telangiectasia protein mutated (ATM) gene encodes ataxia telangiectasia protein, mutant form of which impairs the vitally important function of DSB repair of DNA and cell cycle; increased risk of BC is also reported in carriers of ATM gene though not much significantly.[28] It was demonstrated that the polymorphism of 5557G>A at exon 39 of this gene is more frequent in BC patients with significant difference compared to the control group.[29] In certain populations, astrocytoma development is also found in the 5557G>A in conjunction with some other genes.[28]

Hereditary breast ovarian cancer by BRCA1/2

Most commonly influencing genes in breast/ovarian carcinomas are BRCA1 and BRCA2. BRCA1 and BRCA2 genes are found to be associated with more aggressive and higher-grade breast tumours.[29] Cancer progression elevates up to 80% by involvement of BRCA1/2 genes.[30] Loss of function of BRCA1/2 is also involved in elevated risk of ovarian, prostate, pancreatic and male BC.[31] Moreover, there is specific pattern of genetic aberrations associated with BRCA1/2 that demonstrate specific behaviour during tumorigenesis. Differential expression levels of Proto-oncogene (P53), transcription factor (MYB) human epidermal growth factor receptor-2 (HER2) and cyclin D1 (CCND1) was found, while compared BC tumour cells with BRCA1 defective variant with sporadic BC tumour cells.[32]

Historical Account of BRCA1/2 genes

BRCA1/2 genes were discovered nearly 20 years ago after which lots of progress has been witnessed. A major landmark regarding its treatment was coined after about 2 decades of discovery of BRCA when the United States Food and Drug Administration (FDA) approved olaparib as the chemo-therapeutic agent for ovarian carcinoma patients with BRCA-positive mutation. Major milestones are the pre-clinical studies for testing olaparib drug therapy in 2005, ruling out patents right of Myriad Lab for genetic testing in 2013 and approval of olaparib by FDA for BRCA mutant-positive patients.[33] In recent years, new treatment option specified olaparib tablets for BRCA mutants with HER2-negative BC.[34]

BRCA1 Nomenclature and Resources

Homo sapiens breast cancer susceptibility gene 1 is also known as familial breast/ovarian cancer gene 1, located

on chromosome number 17 q-21 possessing gene identification (ID) 672. This gene has various other synonyms such as BRCC1, PSCP, IRIS and RNF53 (Alias symbols of BRCA1). Ensemble ID is ENSG00000012048. This gene is present on the negative strand. Genomic coordinates are: 17: 43045678 to 43124096. There are 27 different three-dimensional (3D) structures which can be viewed on Catalogue of Somatic Mutations In Cancer-3D (COSMIC-3D). So far, 47341 unique samples have been recorded. Alternate assembly of transcripts is annotated by BRCA1_ENST0000047118. Various sequences for this gene can be viewed on complementary DNA (cDNA-ENST00000357654). Transcript and protein alignment can be observed at ENST00000357654+BRCA1. Mutation data and drug sensitivity can be seen on PF-4708671. This Information about gene can be viewed on official website of National Centre for Biotechnology Information.[35]

Besides NCBI, external links and resources include genome browsers Ensemble and UCSC. Link related to bioinformatics resources of BRCA1 is Online Mendelian Inheritance in Man (OMIM). Transcript ID is ENST00000357654. Copy Number Analysis (CONAN) command would be used for observation of copy number. Gene name in Atlas of Genetic Oncology is BRCA1, while human genome nomenclature (HGNC) ID is 1100, detail of such information can be viewed by browsing at home page of Human Genome Nomenclature Committee.[36]

Structure of BRCA1

BRCA1 was mapped by Hall et al.[37] in 1990 and was found to be linked to Chromosome[17] q-21 by linkage method. The structure was further revealed by Miki et al.[17] in 1994 through cloning method since they observed the strong predisposition in five out of eight kindred who were probable candidates to segregate BRCA1 allele. In the mentioned initial investigative study for cloning this gene, 1 base pair insertion, 11 base pairs deletion,

missense substitution and formation of stop codon was observed. Discovery of the gene has greatly revolutionised the understanding of BC biology. The gene comprises of 24 exons (Figure-1). The protein products encoded by BRCA1 consist of 1863 amino acids.[17] More than 40% of BRCA1 gene is composed of Alu repeats sequences and some other repeated sequences with low frequency.[38] The gene is spread over 117kbp of genome with exon 11 covering 3426 base pairs, making it the biggest human exon.[39]

BRCA1 gene produces three isoforms by alternative splicing; one including all exons, other formed by skipping of exon 11, and the third includes only 117 bases of exon 11 along with rest of all exons respectively called full isoform, △11 isoform and △11q isoforms; the last one is also called in-frame of BRCA1 intron 11 splice(IRIS) form consisting of 1399 amino acids.[40,41] BRCA1 full isoform consists of many conserved crucially functional domains, including ring domain on N terminus, two nuclear localisation regions and BRCA1 C Terminus (BRCT) domain on the C terminus.[42] BRCA1 is one of the important genes belonging to tumour suppressor class categorised as "guardians of genome", therefore protein products have some important DNA-binding domains for the regulation of DNA repair pathways and apoptosis.[43] There are around 15 other target genes, including BRCA2, ATM, interacting protein (CtIP) and Tumor Protein P53which are harboured by BRCA1 for such regulation and, thus, it is said to be the key regulator for the maintenance of genomic integrity by control on DNA repair and apoptosis.[44]

Summary of BRCA1 Functions

The wild type BRCA1 gene products play crucial role in the control of checkpoints of the cell cycle.[45] Most of the products of mutations consist of truncated protein. Functional features of BRCA1 have been investigated by gene knockout experiments using different organism
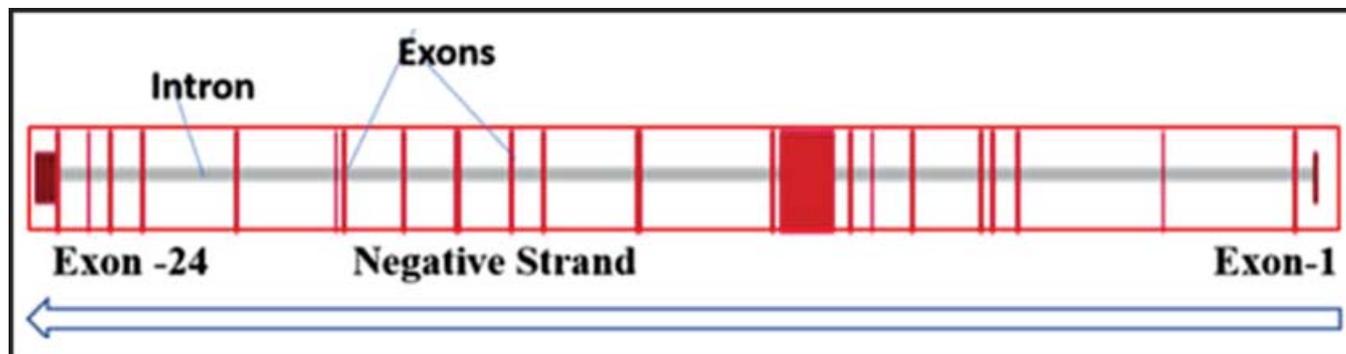
Figure-1: Graphical representation of breast cancer susceptibility gene-1 (BRCA1) showing exon-intron arrangement and relative length represented by width of bars. Reproduced from the Atlas of Genetics and Cytogenetics in Oncology and Haematology. http://atlasgeneticsoncology.org/Genes/png/BRCA1.png [Accessed 25 April, 2018].

models.[46] Crucially vital role of BRCA1 could be explained by its association with various important DNA repair proteins like RAD1, P53, RNA helicase, holoenzyme of RNA polymerase II, terminal Binding Protein (CtBP) interacting protein, BRCA1 associated RING Domain 1 (BARDI),MYC Proto-Oncogene, Basic helix-loop-helix (BHLH) Transcription Factorc-myc and BRCA2.[47] All these associated proteins strongly predict the important role of this gene in transcriptional transactivation, DNA repair and control on cell cycle.[29] The BRCA1 plays its role in DNA DSB repairs, mediated by homologous-recombination.[48] Thus, summarising the functions of BRCA1, it can be said to act as a vehicle for converging cell regulatory proteins. Mutations in BRCA1, therefore, affect the composition of formation of complexes which results in deregulation of cellular repair processes and, eventually, the development of malignant tumours.

Some vitally important functions of BRCA1 gene need to be fully kept in mind.

Role in control of cell division: G2-M checkpoint activation

It has been found that DNA mismatch repair (MMR) is involved in the chemotherapeutic response to some drugs like 6-thioguanine (6-TG) during cancer treatments in human beings.[49] Consistent resistance to 6-TG was observed in some MMR-deficient cells which reveals the G2-M checkpoint arrest, decreased rate of apoptosis and more resilience to thioguanine treatment and genotoxicity.[50] Yamane et al.[51] in 2007 investigated isogenic human BC cell line models, including a mutated BRCA1 cell line thyroid carcinoma, Hurthle cell (HCC-1937), and concluded that BRCA1 mutated cells showed more resistance to 6-TG than to BRCA1-positive cells and almost a complete loss of G2-M cell cycle checkpoint response induced by 6-TG.

Role in differentiation and development

BRCA1 mutated cells impair the process of differentiation and enhance proliferation in mammary epithelial cells. Furuta et al.[52] in 2006 demonstrated the direct functioning of BRCA1 in the process of differentiation and development of acinus formation in mammary epithelial cells by using 3D in-vitro culture system. They concluded that BRCA1-deficient cells impair acinus formation and enhance proliferation by RNA interference. Recently, a similar kind of observation about BRCA1-mediated DNA repair has been made in humans regarding its involvement in the stabilisation of differentiation state inmucosae-associated epithelial chemokine (MEC) whose previous name was spliceosome factor which was then changed to DNA replication regulator and spliceosome

factor (SMU1).[53] In another investigation, BRCA1 wild type cell lines were compared with haplo-insufficient BRCA1 pathogenic variant which resulted in high-impact deficiency in the process of differentiation rendering cells more prone to malignancy.[54]

Genome instability

BRCA1 gene is strongly associated with HR-mediated DNA repair, thus mutant gene products develop genomic instability as Tirkkonen et al.[55] in 1997 observed high degree of aneuploidy in BRCA1 mutant cells compared to non-mutant cells. BC susceptibility gene 1 requires RAD1 during the assembly of subnuclear components, thus impaired functioning lead to genomic instability.[56] BRCA1 was also explored to play a role in other DNA repair mechanisms like non-homologous end joining, nucleotide-excision repair, and base excision repair as it has an association with a large number of important proteins.[49]

Angiogenesis

It was found during one of the investigations that the removal of BRCA1/interacting protein (CtIP) complex from Angiopoietin 1 (ANG1) promotor, accelerates the tumour growth in mammary tissues with noticeable vascularisation.[52]

Induction of apoptosis-escaping from programmed cell death

Inactivation of extracellular signal-regulated kinase 2 (ERK1/2) functioning during BRCA1-driven apoptosis was explored by Yan et al.[57] thus, indicating its role in escaping programmed cellular death. BRCA1-induced apoptosis was also found to be involved in activation of Jun N terminal kinase (JNK), Cell Surface Death Receptor (Fas-l/Fas) and caspases 8/9.[57]

Mis-localisation of cytosol to promote metastasis and cellular invasion

In-vitro studies on genetically-induced BRCA1 mutant human cells revealed the cytosolic mis-localisation and increased cellular invasion activity.[58] The feature of cytosol mis-localisation related to specific BRCA1 mutation could be utilised as a biomarker to predict the disease status, especially metastasis. BRCA1-deficient condition also relates to increased metastasis in brain tissue and shows DNA damage induction and sensitivity to Poly ADP-ribose polymerase (PARP). PARP is a family of proteins involved in a number of cellular processes involving mainly DNA repair and programmed cell death polymerase inhibitor.[59]

Changes in the Bioenergetics

Wild type BRCA1 gene is also strongly associated with bioenergetics of the cell. In the year 2014, Jackson et al.[60] investigated the role of BRCA1 gene product in different tissues, including breast tissue, by using translational approach. They found many isoforms of BRCA1 in human and mice muscle cells. In response to exercise, increased interaction between phosphorylated acetyl CoA carboxylase and BRCA1 was observed. The same study found that the decrease in the amount of BRCA1 resulted in decreased consumption of mitochondrial oxygen and increased production of reactive oxygen. Thus, BRCA1 plays a vital role in regulation of metabolic activities. Moreover, BRCA1 acts like a potentially important biomarker for insulin like growth factor 1 (IGF1) like growth-factor targeted therapy for BC.[61] The over-expression of the BRCA1 gene increases anti-oxidation activity in breast carcinomic malignancies.[62]

BRCA2 Nomenclature and Resources

Homo sapiens BRCA2 is located on chromosome 13 q-13.1, also known as familial breasts and/or ovarian cancer gene 2. Its gene ID is 675. This gene has various other synonyms, which are BRCC2, FAD, FAD1, FACD, FANCD1, FANCD and RPOVCA2 (Fanconi anemia, complementation group D1 alias names of BRCA1/BRCA2-containing complex, subunit 2).

Ensemble ID is ENSG00000139618. The gene is present on the positive strand. Genomic coordinates are 13:32316461 to13: 32398770(+). There are only two different 3D structures which can be viewed on Catalogue of Somatic Mutations In Cancer-3D (COSMIC-3D). BRCA2 possess numerous copies of motifs consisting of 70 amino acids named as activator of Rho GEF and GTPase (BRC). These motifs facilitate bonding of RAD51 to become functionally active in DNA repair. Loss of heterozygosity in BRCA2 tumours indicates its inclusion in class of TSGs.

Human genome Nomenclature ID of BRCA2 is 1101. The database for BRCA2 is available on official website of National Centre of Biotechnology Information.[63]

Structure of BRCA2

The BRCA2 gene was first identified by Wooster[64] in 1995 by using positional cloning method. This large gene comprises 27 exonic regions coding 3418 amino acids (Figure-2).[65] This gene is categorised as TSG by further investigations on familial BC data.[66] Various isoforms have been identified by exploring splice sites.[67] BRCA2 mainly involves maintaining genomic integrity but also has important regulatory significance.[68]

Summary of BRCA2 functions

Multiple range of functions has been explored for BRCA2 during the functional assays, a TSG with vital role in chromosomal stability. It controls the RAD51 during DNA repair by homologous recombination (HR) and mitotic advancement at G2/M checkpoint, spindle assembly and is also involved in cytokinesis.[69] Loss of functions account for chromosomal aberrations in carcinoma cells driving neoplastic alterations and mutagenesis.[29] BRCA2 also acts as modulator of process of proliferation, migration and cellular invasion by controlling Matrix Metallopeptidase 9 (MMP-9) expression.[70] Duplication of centromere, gametogenesis, replication of telomeres, control on cytokinesis and regulation of transcriptional products are some other important processes where involvement of BRCA2 is important.[70]

The following are some important functions carries out by BRCA2 gene.

Response to Replication Fork

Defects in BRCA2 functioning lead to both collapsed and stalled replication fork.[72] The BRCA2 and RAD51 genes are
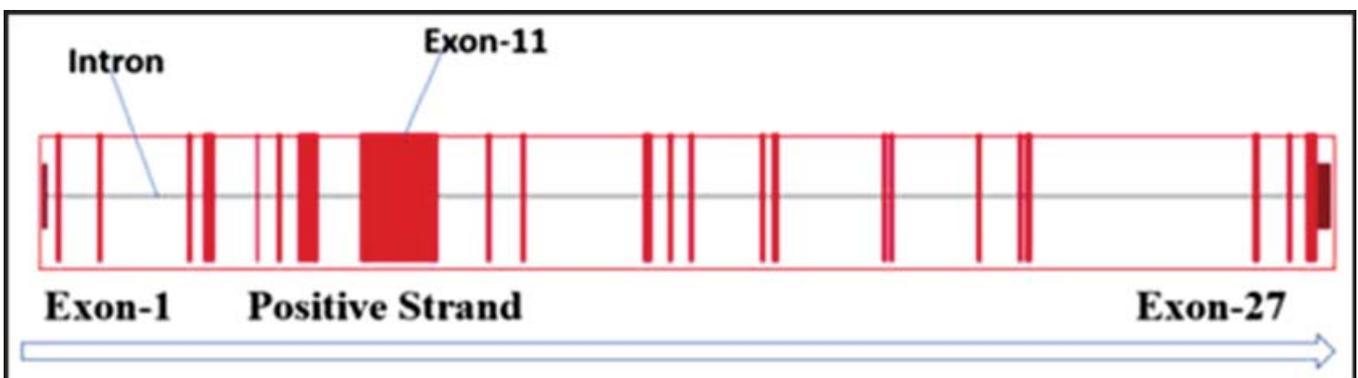
Figure-2: Graphical representation of breast cancer susceptibility gene-2 (BRCA2) showing exon-intron arrangement. http://atlasgeneticsoncology.org/Genes/png/BRCA2.png [Accessed 25 April, 2018]

involved in the stabilisation of replication-stalled fork in single-stranded DNA. This function is not dependent on the activity of nuclease multiple response expansion 1(MRE1).[73] Moreover, BRCA2 shelters the nascent single-stranded DNA from degradation at the position of the stalled fork.[74]

Role in Programmed cell death

Guaragnella et al.[75] in 2014 used sensitised acid-induced apoptotic yeast cells and explored that the BRCA2 silencing causes decreased expression of anoikis. Cellular adhesion to matrix protein in extracellular environment is a necessary component of cell survival, and failure of such adhesion results in a specific type of apoptosis called anoikis. The process of anoikis is an important mechanism to prevent the dead cell from being misplaced, and plays a vital role in averting metastasis.[76] Thus, the function of BRCA2 is modular of anoikis with the involvement of Receptor Tyrosine Kinase (ROS).[75]

Conclusion

There are dozens of genes involved in breast and ovarian cancers among which BRCA1/2 genes are the most dominant contributors. The knowledge of structural and functional features of these genes plays a pivotal role in research plans to explore new horizons in cancer genetics and BRCA1/2 spectra of any specific population. Moreover, enhanced knowledge of cancer genetics and the involvement of BRCA1/2 genes in breast carcinoma would also augment the direction of research to improve treatment strategies.

Disclaimer: The text is part of a PhD dissertation.

Conflict of Interest: None.

Source of Funding: None.

References

1. Kastan MB. DNA damage responses: mechanisms and roles in human disease: 2007 G.H.A. Clowes Memorial Award Lecture. Mol Cancer Res 2008;6:517-24. doi: 10.1158/1541-7786.MCR-08-0020.
2. Bernstein C, Holubec H, Bhattacharyya AK, Nguyen H, Payne CM, Zaitlin B, et al. Carcinogenicity of deoxycholate, a secondary bile acid. Arch Toxicol 2011;85:863-71. doi: 10.1007/s00204-011-0648-7.
3. Malkin D. Li-fraumeni syndrome. Genes Cancer 2011;2:475-84. doi: 10.1177/1947601911413466.
4. Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. Science 2007;318:1108-13. DOI: 10.1126/science.1145720
5. Balaguer F, Link A, Lozano JJ, Cuatrecasas M, Nagasaka T, Boland CR, et al. Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. Cancer Res 2010;70:6609-18. doi: 10.1158/0008-5472.CAN-10-0622.
6. Weinberg RA. The Biology of Cancer, 2nd ed. New York, USA: Garland Science, Taylor & Francis Group, LLC; 2014: pp 876.
7. Ponder BA. Cancer genetics. Nature 2001;411:336-41. DOI: 10.1038/35077207
8. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013;339:1546-58. doi: 10.1126/science.1235122.
9. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature 2001;411:342-8. DOI: 10.1038/35077213
10. Sedic M, Skibinski A, Brown N, Gallardo M, Mulligan P, Martinez P, et al. Haploinsufficiency for BRCA1 leads to cell-type-specific genomic instability and premature senescence. Nat Commun 2015;6:7505. doi: 10.1038/ncomms8505.
11. Kinzler KW, Vogelstein B. Landscaping the cancer terrain. Science 1998;280:1036-7. DOI: 10.1126/science.280.5366.1036
12. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 2012;481:157-63. doi: 10.1038/nature10725.
13. Zhou K, Liu M, Cao Y. New Insight into microRNA Functions in Cancer: Oncogene-microRNA-Tumor Suppressor Gene Network. Front Mol Biosci 2017;4:e46. doi: 10.3389/fmolb.2017.
14. Khatami F, Larijani B, Heshmat R, Keshtkar A, Mohammadamoli M, Teimoori-Toolabi L, et al. Meta-analysis of promoter methylation in eight tumor-suppressor genes and its association with the risk of thyroid cancer. PLoS One 2017;12:e0184892. doi: 10.1371/journal.pone.0184892.
15. Apostolou P, Fostira F. Hereditary breast cancer: the era of new susceptibility genes. Biomed Res Int 2013;2013:e747318. doi: 10.1155/2013/747318.
16. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature 2009;458:719-24. doi: 10.1038/nature07943.
17. 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. DOI: 10.1126/science.7545954
18. 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. DOI: 10.1038/378789a0
19. Karami F, Mehdipour P. A comprehensive focus on global spectrum of BRCA1 and BRCA2 mutations in breast cancer. Biomed Res Int 2013;2013:e928562. doi: 10.1155/2013/928562.
20. Corso G, Intra M, Trentin C, Veronesi P, Galimberti V. CDH1 germline mutations and hereditary lobular breast cancer. Fam Cancer 2016;15:215-9. doi: 10.1007/s10689-016-9869-5.
21. Kaurah P, MacMillan A, Boyd N, Senz J, De Luca A, Chun N, et al. Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer JAMA 2007;297:2360-72. DOI: 10.1001/jama.297.21.2360
22. Chen J, Lindblom A. Germline mutation screening of the STK11/LKB1 gene in familial breast cancer with LOH on 19p. Clin Genet 2000;57:394-7. DOI: 10.1034/j.1399-0004.2000.570511.
23. Rosselli-Murai LK, Yates JA, Yoshida S, Bourg J, Ho KKY, White M, et al. Loss of PTEN promotes formation of signaling-capable clathrin-coated pits. J Cell Sci 2018;131:208926. doi: 10.1242/jcs.208926.
24. Xu C, Fillmore CM, Koyama S, Wu H, Zhao Y, Chen Z, et al. Loss of Lkb1 and Pten leads to lung squamous cell carcinoma with elevated PD-L1 expression. Cancer Cell 2014;25:590-604. doi: 10.1016/j.ccr.2014.03.033.
25. Lukas C, Bartkova J, Latella L, Falck J, Mailand N, Schroeder T, et al. DNA damage-activated kinase Chk2 is independent of proliferation or differentiation yet correlates with tissue biology. Cancer Res 2001;61:4990-3.
26. Zannini L, Delia D, Buscemi G. CHK2 kinase in the DNA damage response and beyond. J Mol Cell Biol 2014;6:442-57. doi: 10.1093/jmcb/mju045.
27. Wong MW, Nordfors C, Mossman D, Pecenpetelovska G, Avery-Kiejda KA, Talseth-Palmer B, et al. BRIP1, PALB2, and RAD51C mutation analysis reveals their relative importance as genetic

susceptibility factors for breast cancer. Breast Cancer Res Treat 2011;127:853-9. doi: 10.1007/s10549-011-1443-0.

28. Gao L, Li F, Dong B, Zhang J, Rao Y, Cong Y, et al. Inhibition of STAT3 and ErbB2 suppresses tumor growth, enhances radiosensitivity, and induces mitochondria-dependent apoptosis in glioma cells. Int J Radiat Oncol Biol Phys 2010;77:1223-31. doi: 10.1016/j.ijrobp.2009.12.036.

29. Venkitaraman AR. Cancer suppression by the chromosome custodians, BRCA1 and BRCA2. Science 2014;343:1470-5. doi: 10.1126/science.1252230.

30. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994;343:692-5. DOI: 10.1016/s0140-6736(94)91578-4

31. Cavanagh H, Rogers KM. The role of BRCA1 and BRCA2 mutations in prostate, pancreatic and stomach cancers. Hered Cancer Clin Pract 2015;13:e16. doi: 10.1186/s13053-015-0038-x

32. Cass JD, Varma S, Day AG, Sangrar W, Rajput AB, Raptis LH, et al. Automated Quantitative Analysis of p53, Cyclin D1, Ki67 and pERK Expression in Breast Carcinoma Does Not Differ from Expert Pathologist Scoring and Correlates with Clinico-Pathological Characteristics. Cancers (Basel) 2012;4:725-42. doi: 10.3390/cancers4030725.

33. Walsh CS. Two decades beyond BRCA1/2: Homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy. Gynecol Oncol 2015;137:343-50. doi: 10.1016/j.ygyno.2015.02.017.

34. Le D, Gelmon KA. Olaparib tablets for the treatment of germ line BRCA-mutated metastatic breast cancer. Expert Rev Clin Pharmacol 2018;11:833-9. doi: 10.1080/17512433.2018.1513321.

35. BRCA1. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. [Online] 2004 [Cited 2018 September 04]. Available from URL: https://www.ncbi.nlm.nih.gov/gene/672

36. HGNC Database, HUGO Gene Nomenclature Committee (HGNC), European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, Cambridge CB10 1SD, United Kingdom. [Online] [Cited 2018 September 04]. Available from URL: https://www.genenames.org/data/gene-symbol-report/#!/hgnc_id/HGNC:1100

37. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990;250:1684-9. DOI: 10.1126/science.2270482

38. Zhang J, Fackenthal JD, Huo D, Zheng Y, Olopade OI. Searching for large genomic rearrangements of the BRCA1 gene in a Nigerian population. Breast Cancer Res Treat 2010;124:573-7. doi: 10.1007/s10549-010-1006-9.

39. Raponi M, Smith LD, Silipo M, Stuani C, Buratti E, Baralle D. BRCA1 exon 11 a model of long exon splicing regulation. RNA Biol 2014;11:351-9. doi: 10.4161/rna.28458.

40. Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, et al. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Mol Cell 1999;3:389-95. DOI: 10.1016/s1097-2765(00)80466-9

41. Tammaro C, Raponi M, Wilson DI, Baralle D. BRCA1 exon 11 alternative splicing, multiple functions and the association with cancer. Biochem Soc Trans 2012;40:768-72. DOI: 10.1042/BST20120140.

42. O'Donovan PJ, Livingston DM. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis 2010;31:961-7. doi: 10.1093/carcin/bgq069.

43. Cable PL, Wilson CA, Calzone FJ, Rauscher FJ, Scully R, Livingston DM, et al. Novel consensus DNA-binding sequence for BRCA1 protein complexes. Mol Carcinog 2003;38:85-96. DOI: 10.1002/mc.10148

44. Li S, Ting NS, Zheng L, Chen PL, Ziv Y, Shiloh Y, et al. Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response. Nature 2000;406:210-5. DOI: 10.1038/35018134

45. Deng CX, Brodie SG. Roles of BRCA1 and its interacting proteins. Bioessays 2000;22:728-37. DOI: 10.1002/1521-1878(200008)22:8<728::AID-BIES6>3.0.CO;2-B

46. Brodie SG, Deng CX. BRCA1-associated tumorigenesis: what have we learned from knockout mice? Trends Genet 2001;17:18-22. DOI: 10.1016/s0168-9525(01)02451-9

47. Deng CX. BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. Nucleic Acids Res 2006;34:1416-26. DOI: 10.1093/nar/gkl010

48. Prakash R, Zhang Y, Feng W, Jasin M. Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. Cold Spring Harb Perspect Biol 2015;7:a016600. doi: 10.1101/cshperspect.a016600.

49. You C, Dai X, Yuan B, Wang Y. Effects of 6-thioguanine and S6-methylthioguanine on transcription in vitro and in human cells. J Biol Chem 2012;287:40915-23. doi: 10.1074/jbc.M112.418681.

50. Yan T, Berry SE, Desai AB, Kinsella TJ. DNA mismatch repair (MMR) mediates 6-thioguanine genotoxicity by introducing single-strand breaks to signal a G2-M arrest in MMR-proficient RKO cells. Clin Cancer Res 2003;9:2327-34.

51. Yamane K, Schupp JE, Kinsella TJ. BRCA1 activates a G2-M cell cycle checkpoint following 6-thioguanine-induced DNA mismatch damage. Cancer Res 2007;67:6286-92. DOI: 10.1158/0008-5472.CAN-06-2205

52. Furuta S, Wang JM, Wei S, Jeng YM, Jiang X, Gu B, et al. Removal of BRCA1/CtIP/ZBRK1 repressor complex on ANG1 promoter leads to accelerated mammary tumor growth contributed by prominent vasculature. Cancer Cell 2006;10:13-24. DOI: 10.1016/j.ccr.2006.05.022

53. Wang H, Bierie B, Li AG, Pathania S, Toomire K, Dimitrov SD, et al. BRCA1/FANCD2/BRG1-Driven DNA Repair Stabilizes the Differentiation State of Human Mammary Epithelial Cells. Mol Cell 2016;63:277-92. doi: 10.1016/j.molcel.2016.05.038.

54. Feilotter HE, Michel C, Uy P, Bathurst L, Davey S. BRCA1 haploinsufficiency leads to altered expression of genes involved in cellular proliferation and development. PLoS One 2014;9:e100068. doi: 10.1371/journal.pone.0100068.

55. Tirkkonen M, Johannsson O, Agnarsson BA, Olsson H, Ingvarsson S, Karhu R, et al. Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. Cancer Res 1997;57:1222-7.

56. Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK. The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. J Biol Chem 2000;275:23899-903. DOI: 10.1074/jbc.C000276200

57. Yan Y, Haas JP, Kim M, Sgagias MK, Cowan KH. BRCA1-induced apoptosis involves inactivation of ERK1/2 activities. J Biol Chem 2002;277:33422-30. DOI: 10.1074/jbc.M201147200

58. Santivasi WL, Wang H, Wang T, Yang Q, Mo X, Brogi E, et al. Association between cytosolic expression of BRCA1 and metastatic risk in breast cancer. Br J Cancer 2015;113:453-9. doi: 10.1038/bjc.2015.208.

59. McMullin RP, Wittner BS, Yang C, Denton-Schneider BR, Hicks D, Singavarapu R, et al. A BRCA1 deficient-like signature is enriched in breast cancer brain metastases and predicts DNA damage-induced poly (ADP-ribose) polymerase inhibitor sensitivity. Breast Cancer Res 2014;16:e25. doi: 10.1186/bcr3625.

60. Jackson KC, Gidlund EK, Norrbom J, Valencia AP, Thomson DM,

Schuh RA, et al. BRCA1 is a novel regulator of metabolic function in skeletal muscle. J Lipid Res 2014;55:668-80. doi: 10.1194/jlr.M043851.

61. Cohen-Sinai T, Cohen Z, Werner H, Berger R. Identification of BRCA1 As a Potential Biomarker for Insulin-Like Growth Factor-1 Receptor Targeted Therapy in Breast Cancer. Front Endocrinol (Lausanne) 2017;8:e148. doi: 10.3389/fendo.2017.00148.

62. Bae I, Fan S, Meng Q, Rih JK, Kim HJ, Kang HJ, et al. BRCA1 induces antioxidant gene expression and resistance to oxidative stress. Cancer Res 2004;64:7893-909. DOI: 10.1158/0008-5472.CAN-04-1119

63. BRCA2. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. [Online] 2004[Cited 2018 September 04]. Available from URL: https://www.ncbi.nlm.nih.gov/gene/675

64. 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. DOI: 10.1038/378789a0

65. 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.

66. Gudmundsson J, Johannesdottir G, Bergthorsson JT, Arason A, Ingvarsson S, Egilsson V, et al. Different tumor types from BRCA2 carriers show wild-type chromosome deletions on 13q12-q13. Cancer Res 1995;55:4830-2.

67. Speevak MD, Young SS, Feilotter H, Ainsworth P. Alternatively spliced, truncated human BRCA2 isoforms contain a novel coding exon. Eur J Hum Genet 2003;11:951-4. DOI: 10.1038/sj.ejhg.5201063

68. Marmorstein LY, Ouchi T, Aaronson SA. The BRCA2 gene product functionally interacts with p53 and RAD51. Proc Natl Acad Sci U S A 1998;95:13869-74. DOI: 10.1073/pnas.95.23.13869

69. Rosenthal CK. Brca2 in abscission. Nature Cell Biology 2012;14:792.

70. Moro L, Arbini AA, Yao JL, di Sant'Agnese PA, Marra E, Greco M. Loss of BRCA2 promotes prostate cancer cell invasion through up-regulation of matrix metalloproteinase-9. Cancer Sci 2008;99:553-63. doi: 10.1111/j.1349-7006.2007.00719.x.

71. Badie S, Escandell JM, Bouwman P, Carlos AR, Thanasoula M, Gallardo MM, et al. BRCA2 acts as a RAD51 loader to facilitate telomere replication and capping. Nat Struct Mol Biol 2010;17:1461-9. doi: 10.1038/nsmb.1943.

72. Kim TM, Son MY, Dodds S, Hu L, Hasty P. Deletion of BRCA2 exon 27 causes defects in response to both stalled and collapsed replication forks. Mutat Res 2014;766-767:66-72. doi: 10.1016/j.mrfmmm.2014.06.003.

73. Sirbu BM, Couch FB, Feigerle JT, Bhaskara S, Hiebert SW, Cortez D. Analysis of protein dynamics at active, stalled, and collapsed replication forks. Genes Dev 2011;25:1320-7. doi: 10.1101/gad.2053211.

74. Schlacher K, Christ N, Siaud N, Egashira A, Wu H, Jasin M. Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. Cell 2011;145:529-42. doi: 10.1016/j.cell.2011.03.041.

75. Guaragnella N, Marra E, Galli A, Moro L, Giannattasio S. Silencing of BRCA2 decreases anoikis and its heterologous expression sensitizes yeast cells to acetic acid-induced programmed cell death. Apoptosis 2014;19:1330-41. doi: 10.1007/s10495-014-1006-z.

76. Paoli P, Giannoni E, Chiarugi P. Anoikis molecular pathways and its role in cancer progression. Biochim Biophys Acta 2013;1833:3481-98. doi: 10.1016/j.bbamcr.2013.06.026.