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Review Article

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

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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.

Key Words: 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 micro ribonucleic 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 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 chromosome17 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, Δ11 isoform and Δ11q 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 *P53* which 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 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 Factor c-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 in mucosae-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 to 13: 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.[71]

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 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.

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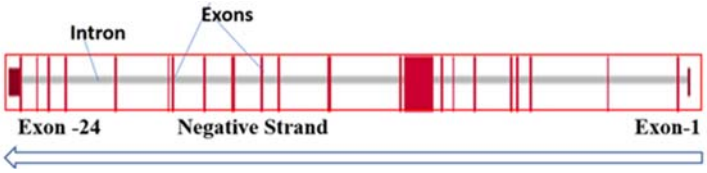

***Figure* 1: Graphical representation of breast cancer susceptibility gene-1 (*BRCA1*) showing exon-intron arrangement and relative length represented by width of bars. Reproduced from the Atlas of Genetics and Cytogenetics in Oncology and Haematology. http://atlasgeneticsoncology.org/Genes/png/BRCA1.png [Accessed 25 April.,2018]**

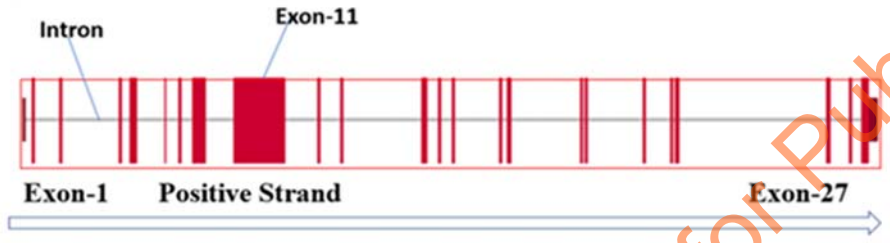

Figure 2: Graphical representation of breast cancer susceptibility gene-2 (*BRCA2*) showing exon-intron arrangement.
http://atlasgeneticsoncology.org/Genes/png/BRCA2.png [Accessed 25 April.,2018]