

The BRCA1 tumor suppressor: potential long-range interactions of the BRCA1 promoter and the risk of breast cancer

Beata Anna Bielinska*

Department of Molecular and Translational Oncology, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Poland

Abstract

Breast cancer is a complex disease with different phenotypes associated with genetic and non-genetic risk factors. An aberrant expression of the BRCA1 tumor suppressor as well as dysfunction of BRCA1 protein caused by germline mutations are implicated in breast cancer aetiology. BRCA1 plays a crucial role in genome and epigenome stability. Its expression is auto regulated and modulated by various cellular signals including metabolic status, hypoxia, DNA damage, estrogen stimulation. The review describes breast cancer risk factors, the BRCA1 gene expression and functions as well as covers the role of long-range genomic interactions, which emerge as regulators of gene expression and moderators of genomic communication. The potential long-range interactions of the BRCA1 promoter (driven by polymorphic variant rs11655505 C/T, as an example) and their possible impact on the BRCA1 gene regulation and breast cancer risk are also discussed.

Introduction

Female breast cancer is one of the most common cancers worldwide with app.1.68 million cases diagnosed annually [1]. It is a complex disease characterized by molecular and phenotypic heterogeneity observed both within populations and intra-individually, within single tumor cells in a spatiotemporal manner, as pointed out by [2]. This intra-individual molecular diversity could be reflective of the mutational history of tumor cells. It is hypothesized that breast cancer can result from clonal expansion of adult stem cells and/or stem progenitor cells, which became cancer stem cells by acquiring tumor initiating capacity [3].

Genetic and non-genetic breast cancer risk factors

A minority of breast cancers (5-10% of all cases) demonstrate familial clustering and have an important genetic component [4]. Familial relative risk (FRR) increases progressively along with the number of affected relatives [5]. Family history is influenced by a number of complex genetic mechanisms, including prenatal effects, mitochondrial variants, sex-linked genes and parental of origin effects exerted by imprinted genes. These may cause its asymmetry and skew the risk of breast cancer towards maternal lineage [6,7]. To date, genetic factors underlying the disease are not fully elucidated.

Rare, high-risk mutations in BRCA1/BRCA2 genes account for less than 20% of FRR. The penetrance of these mutations is incomplete, which suggests there may be modifiers of breast cancer risk among carriers of BRCA1/BRCA2 mutations [4]. To date, a total of 26 and 16 single nucleotide polymorphisms (SNPs), bearing a small risk (in range 1.05-1.26) have been discovered for carriers of these mutations by genome-wide association study (GWAS). Many of them are associated with estrogen receptor (ER) status of the tumor subtype, reviewed by [8]. The risk of breast cancer among BRCA1 mutations carriers is supposed to be influenced by polymorphic variants on the wild-type BRCA1 allele. This could possibly occur through altering the

efficiency of BRCA1 transcription [9]. However, to date the mechanism underlying effect of the BRCA1 promoter variant rs11655505 C>T remains unknown.

Besides high risk BRCA1/BRCA2 mutations, moderate risk mutations such as those found in DNA repair genes (CHEK2, ATM, PALB2) also demonstrate familial clustering. They explain 2-5% of the FRR [10].

Common low risk SNPs identified by GWAS account for even smaller share of FRR. Similarly to variants identified among BRCA1/BRCA2 mutations carriers, common SNPs display differences in genetic susceptibility to ER positive and ER negative tumor subtypes. This suggests that common mechanisms may underlie these phenotypes [8].

In summary, polymorphic variants SNPs identified to date (more than 70), their multiplicative effects (modeled as a polygenic score [PRR]), taken together with mutations in BRCA1, BRCA2, PALB2, ATM, CHEK2 genes account for one-third of FRR.

Based on the data, factors identified to date, do not fully explain genetic susceptibility that is indicated by family history and heritability evidence from studies on monozygotic twins [6,10-13].

Numerous genetic variants with even lower effects on risk, omitted

***Correspondence to:** Beata Anna Bielinska, Department of Molecular and Translational Oncology, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Roentgena Street 5, Warsaw 02-781, Poland, Tel: (48-22)546 2898; E-mail: bbielinska@coi.waw.pl

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by GWAS, might account for missing heritability. A novel approaches has been recently proposed, involving fine SNPs mapping for sub-threshold loci or of reanalyzing and validating GWAS results by using epigenomic signatures [14,15].

Gaining insight into biological function of SNPs is particularly challenging because over 95% of the identified genetic variants fall into non-coding genomic regions and three-quarters of them associate with DNase I-hypersensitive sites. This suggests that they lie within regulatory elements, known to establish long-range contacts and affects target genes located distally even several megabases away. Recently, a number of regulatory SNPs were assigned to their target in breast cancers, when SNPs long-range contacts were taken into account. This was done through linking GWAS and Hi-C analyses (whole genome conformation analysis based on proximity ligation followed by high-throughput sequencing), reviewed by [16].

Especially noteworthy is the evidence, that the majority of breast cancer cases (90-95%) occur sporadically [17]. This may be linked to many factors including gender, age, reproductive and hormonal history, environmental exposure and/or life style (alcohol intake, tobacco smoking, diet habits (specifically high fat diet and toxine exposure) and other stress factors [8,18,19]. Interestingly, several disorders such as obesity and associated metabolic syndrome (including diabetes) are reported to be related to breast cancer risk [20,21]. Similarly to the risk of breast cancer, the risk of obesity and metabolic disorders may be sexually dimorphic- presumably modulated by gonadal hormones and by sex chromosome status (XX, XY) [22]. The broad spectrum of breast cancer risk factors and related disorders has been comprehensively discussed by [8,18].

BRCA1ness in sporadic breast cancers

BRCA1 tumor suppressor is implicated in aetiology of both familial and sporadic breast cancer. Almost 33% of non-familial, invasive sporadic breast cancer either lack or have a reduced expression of BRCA1 (due to somatic alternation or epigenetic silencing) and share the familial-BRCA1 mutated tumor's phenotype, as reviewed recently by [23]. The loss of BRCA1 or its dysfunction is presumably the critical step for the formation of the basal-like subtype of breast cancer (BBC), a high-grade, aggressive tumor with lymphocytic infiltrates. The minority of cases (10-30%) show hypermethylation of the BRCA1 promoter [24]. Their transcriptomic signature is characterized by expression of genes mostly active in breast myoepithelial layer (basal-layer) [23], whereas their epigenetic characteristic is similar to those observed in embryonal stem cells (esc), including overexpression of pioneer transcription factors (Nanog, SOX2 and c-Myc) and under-expression of Polycomb-regulated genes, as reported by [25].

A significant portion of sporadic breast cancer are estrogen receptor negative (ER-), similarly to familial BRCA1 mutated tumors. They tend to lack progesterone receptor (PR-), ERB-2 oncogen (HER2) and display triple-negative TN phenotype. Molecular characteristic of these tumors also includes genomic and chromosomal instability as stated by [26,27]. Chromosome X gains were observed in neoplastic transformation of male epithelial cells [28], whereas epigenetic instability/loss of inactive X chromosome frequently occurs in female basal breast cancer cases [29]. Similarly to other solid tumors, basal like tumors demonstrate heterogeneity of their tumoral microenvironment, including intra-tumoral level of oxygen, nutrient and pH [30,31].

Hypoxia stress has been found to induce down-regulation of BRCA1 expression and this partially explains repression of the BRCA1

gene observed in sporadic breast cancers [32]. Due to the cellular role of BRCA1, its deficiency may contribute to genomic instability and predispose cells to high risk of malignant transformations. Furthermore, the expression of BRCA1 has been reported to be required for differentiation of breast stem cells, specifically luminal progenitor cell (ER-negative) to mature luminal cells (ER-positive). A loss of BRCA1 may result in the accumulation of genetically unstable breast stem cells, which presumably underlie the aetiology of basal-like breast cancers as stated by [33]. Notably, exposure to hypoxia has recently been demonstrated to induce breast cancer stem cells phenotype [34].

Transcriptional regulation of BRCA1 expression

As evidence emerged, that BRCA1 expression is downregulated in sporadic breast tumors many aspects of its regulation has been extensively studied.

The BRCA1 gene is transcribed from the bidirectional promoter for BRCA1 and for lncRNA NBR2 (Neighbor of BRCA1 Number 2) separated by short (approximately 218 bp) intergenic region [35,36].

This transcriptional unit arose in result of segmental duplication during primate evolution [37]. In mice the BRCA1 gene shares bidirectional promoter with the gene Neighbor of BRCA1 Number 1 (NBR1), which encodes autophagic receptor, reportedly having a role in maintenance of cell stemness [38-40].

The choice of transcription start sites at human BRCA1/NBR2 promoter appears to have crucial importance for proper response of the BRCA1 gene to various micro-environmental stimuli, including genotoxic agents, DNA damage, estrogen stimulation and hypoxia. However, so far reported studies have focused more on cancer or transformed cell lines.

Hypoxia, pro-mitogenic activity of estrogens and DNA damage all modulate the cellular redox (NAD/NADH) ratio. As found by [41], an increased redox ratio uni-directionally enhances transcription from the BRCA1 proximal promoter (Figure 1). This occurs through the removal of co-repressors, including CtBP protein, (metabolic sensor

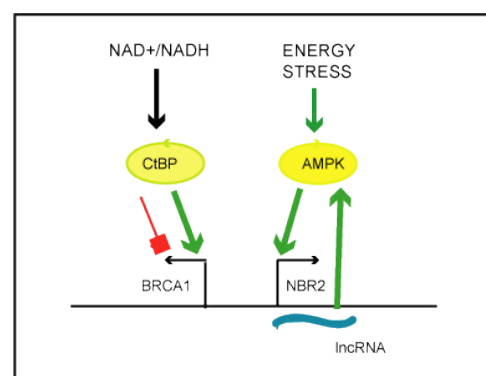


Figure 1. Simplified scheme illustrating the impact of the cellular metabolic states on transcriptional activity of the bidirectional BRCA1 promoter.

According to Di and colleagues, the redox ratio (NAD⁺/NADH) modulates the activity of the metabolic sensor and transcriptional co-repressor, carboxyl-terminal binding protein (CtBP). Increased NAD⁺/NADH ratio cause discharge of CtBP, from the BRCA1 promoter and unidirectional enhancement of transcription from BRCA1 transcription start site.

Liu and colleagues state that energy stress (induced by glucose starvation) activates cellular energy sensor, AMP-activated protein kinase, which by unknown mechanism induces unidirectional transcription of lncRNA from NBR2 transcription start site. lncNBR2 has been found to potentiate activity of AMPK under prolonged stress.

of NAD/NADH ratio), BRCA1, HDAC from the promoter region. In turn, unidirectional transcription of NBR2 lncRNA can be initiated in response to energy stress (glucose starvation), by AMP-activated protein (AMP) kinase, a key sensor of cellular energy status, (Figure 1). NBR2 lncRNA was observed to interact with activated AMPK kinase and it is supposed to amplify and preserve AMPK activity during chronic stress, which results in the repression of anabolic processes of mTORC1 pathway, autophagy promotion, reduction of cell proliferation) [42].

Interestingly, hypoxic stress decreases BRCA1 transcriptional activity not only by modulation of redox ratio but also by dynamic redistributions of E2Fs and pocket proteins at the BRCA1 promoter. BRCA1 expression, which shows cell-cycle related pattern [43,44] is E2F dependent. During normoxia, the two adjacent, conserved E2F sites at the BRCA1 proximal promoter, within intergenic region, are simultaneously occupied by E2F1 transcriptional activator and E2F4/p130 transcriptional repressor. Hypoxia causes p130 dephosphorylation, an increase of the binding of repressive complex E2F4/p130 to the intergenic region and the unidirectional repression of BRCA1 transcription [45].

Extended hypoxia also induces repressive histone modification changes, including decreased H3K4 methylation and leads to persistent epigenetic silencing of the BRCA1 promoter [32].

In turn, oxidative stress stimulates BRCA1 transcription by binding of an activated NRF2 transcription factor (Nuclear factor-erythroid-2p45-related factor 2), the master of redox switch, involved in the Keap1-Nrf2-ARE pathway, to ARE sites (antioxidant response elements) at the proximal BRCA1 promoter [46].

Estrogen has been found stimulate BRCA1 transcription either by non-genomic mechanism (by changes in redox ratio, activation of MAPK cascade) or potentially by recruitment of ER α to the downstream BRCA1 promoter [47]. ER- α dependent activation can be modulated by an aromatic hydrocarbon receptor complex, which binds two consecutive xenobiotic-responsive elements located upstream of the ER- α binding region [48].

The regulation of BRCA1 transcription is further influenced by a number of other transcriptional factors, including, CREB [49], BP53 [50], c-Myc [51].

The bidirectional BRCA1/NBR2 promoter is bound by architectural protein, CTCF transcription factor. The binding protects the promoter region against DNA methylation, maintains its accessibility for transcription factors and is critical for its functionality [52-54].

Mechanism of auto-regulation through co-residence of BRCA1, E2F and Rb1 at the BRCA1 promoter has been also proposed by [55].

According to the authors, BRCA1 transcription is repressed by BRCA1 and upregulated in response to genotoxic stress occurring after the disruption of co-repressors array and dismissal of BRCA1 protein. Subjecting the RB1 gene to genomic imprinting, that favor expression from maternal allele [56] adds another layer to complexity of auto regulation of BRCA1 transcription.

BRCA1 protein and its functions

BRCA1 protein

BRCA1 reportedly interacts with more than 100 of proteins and

has been proposed to act as a scaffold for the assembly of different functional complexes, [57,58].

The BRCA1 C-terminal region contains two BRCT repeats, which constitute a phospho-peptide binding domain, contributing to most of BRCA1 functional interactions, including interactions with signaling kinases ATM, ATR and CHK2. It can be transcriptionally active when ligated with DNA binding domain.

The N-terminal RING domain (with its heterodimeric binding partner, the BRCA1-associated RING-domain protein, BARD1) displays an ubiquitin/ligase activity and functions as a highly active E3 Ub ligase in complexes with E2 ubiquitin (Ub)-conjugating enzymes.

The central, large region (60%) of BRCA1 acts as a scaffold and interacts directly with DNA and proteins. It is required for homologous recombination (HR) and checkpoint functions. Interestingly, it preferentially binds to G-quadruplexes and other non-B-DNA topologically constrained structures, which occur on numerous promoter regions (e.g. C-Myc, KRAS, Kit, TERT genes) and on telomeres [59].

BRCA1 functions

BRCA1 plays a critical role in multiple cellular processes required for genome stability and cellular homeostasis. However, the mechanism of how BRCA1 protein is responsible for increased risk of a breast cancer is not fully understood.

BRCA1 in DNA damage response

The BRCA1 protein functions in a cellular DNA damage response (DDR) network, responding to genotoxic stress. The network detects, signals, repairs DNA/chromatin damage. It also coordinates the repair process with cell cycle progression and cellular metabolism or directs cells to apoptosis. DSBs may occur in result of DNA replication-errors, ionizing radiation and oxidative stress [60]. However, they may also be caused by programmed DSBs arising at specific locations in the genome during meiosis as well as during V(D)J and immunoglobulin heavy chain class switch recombination (CSR) [61]. Among DNA lesions double strand breaks (DSBs) are the most harmful as they may induce severe detriment in DNA and chromatin organization and cause chromosomal translocations. The selection process between the two mechanisms for repairing DSB (error-prone non-homologous end-joining [NHEJ] and homologous recombination [HR]) depends on BRCA1 and on multiple factors, including DNA damage checkpoints, ubiquitination steps and post-translational histone modifications reviewed by [62]. In summary, a master sensor of DSB, Ataxia-Telangiectasia Mutated (ATM) kinase induces histone H2AX phosphorylation cascade and then a process of multi-steps recruitment and assembly of damage signaling and repair factors. It also drives chromatin modifications. The BRCA1 protein once phosphorylated by ATM kinase counteracts inhibitory effect of chromatin barrier, imposed on damage sites by BP53 and then initiates HR by activation of DNA resection. As recently demonstrated, BRCA1-BARD1 E3 Ub ligase causes the repositioning of BP53 over long distance by promoting activity of chromatin remodeler SMARCAD1 (SWI/SNF Matrix-Associated Actin-Dependent Regulator Of Chromatin [63]. As a mediator of ATM signaling, BRCA1 activates DNA damage checkpoints (G2/M phase), reviewed in [64]. It is worth noting, the evidence suggesting that DSB repair pathways are developmentally regulated with HR being crucial in embryonal cells and NHEJ during cell cycles of differentiated cells. DSB repair by HR in primary somatic

cells appears to require BRCA1, whereas ATM kinase is dispensable as stated by [65].

Other molecular processes regulated by BRCA1

BRCA1/BARD1 heterodimer controls microtubule nucleation in spindle assembly and centrosome duplication during mitosis [66].

Apart from repair damage and cell cycle progression control, BRCA1 has other role attributed to its tumor suppression activity. It is supposed to exert global effects on heterochromatin integrity through transcriptional repression of satellite RNA (through ubiquitylation of histone H2A) [67]. Recent reports provide evidence that BRCA1 (in repressive complex with HP1 and DNMT3) may also cause global heterochromatin silencing through ATM dependent DNA methylation [68]. At heterochromatin regions, BRCA1 reportedly participate in protection of DNA replication and it is required for HR at stalled replication forks [69].

Furthermore protein has been found to function as a negative regulator of Polycomb- repressive complex 2 (PCR2), which is important for the maintenance of stem cell pluripotency and suppression of cell differentiation [70].

Its role in transcriptional regulation is complex. BRCA1 regulates transcription by association with basal transcriptional machinery (Polymerase II and Polymerase I holoenzymes) and by interacting and modulating the activity of numerous transcription factors (including p53, c-myc, STAT1, E2F, NF-kB, OCT-1, estrogen, progesterone and androgen receptors), transcriptional co-repressor and co-activators, (including CtBP, Rb- and Rb-associated proteins, HDAC1/2, and p300, HAT), chromatin remodeling complexes (specifically with BRG1-central catalytic ATPase of ATP-dependent chromatin remodeling complexes SWI/SNF [64].

Interestingly BRCA1 has been found at nuclear sub-compartments with transcription machinery (transcription factories), that cluster transcriptionally active or inactive genes [71]. Apart from protection against genotoxic stress, DNA repair proteins at transcription factories are also supposed to control programmed double strand breaks induced by Topoisomerase 2 alfa for proper transcriptional output [72].

Throughout the genome BRCA1 resides at a large number of gene promoters and regulates expression of specific subset of genes in response to genotoxic stress or DNA damage [73,74]. BRCA1 transcriptional complexes regulate activity of pro- and anti-apoptotic genes, genes involved in growth promotion, cell cycle arrest, DNA repair, telomerase and interferon genes described by [58].

Its role in cellular metabolic homeostasis and reprogramming is not completely understood.

BRCA1 is an important negative regulator of anabolic processes promoted by estrogen receptor (ER) and functions in a negative feedback loop, (activating transcription of ER and disrupts estrogen-ER complex) reviewed by [75]. Moreover, it modulates IGF1/PIK3/Akt pathway, (by transcriptional regulation of IGF1 as well as interaction with AKT), [76,77] and fatty acid synthesis (maintaining acetyl-CoA-carboxylase in an inactive state) [78]. BRCA1 has been also observed to regulate NRF2 dependent antioxidant signaling and hypoxia response [binding and stabilizing NRF2 transcription factor [79] and hypoxia-inducible factor-1 α [HIF-1 α] [80], respectively].

Recent metabolomics and transcriptomic data further suggests that BRCA1 can cause reversion of aerobic glycolysis (known as a Warburg effect) in breast cancer cells [81].

Long-range interactions and regulation of genome functions

Genome integrity is accomplished through regulation of DNA replication and genes expression in the three dimensional nuclear spaces.

Genomic long-range interactions (>10kb) are integral for 3D genome organization. As proposed by [82], they can moderate of communication along chromosome (in cis-) and between chromosomes (in trans-). Bringing distant regulatory elements (promoters, enhancers) into spatial proximity allows for effective control of gene expression.

Genomic contacts are established partially by architectural proteins (with key role of CTCF insulator protein and cohesin) and are accompanied by looping out the intervening sequence, [83]. The interactions may be influenced by various chromatin features, protein cofactors and complexes, Mediator, DNA methylation and local RNA transcription reviewed in [84]. Interestingly, basal transcription machinery is known to be recruited by CTCF and cohesin [83,85].

Long-range interactions, detected by Hi-C on chromosomes during interphase are observed as dynamically formed neighborhoods (encompassing app. 400-500 MB), referred to as Topologically Associated Domains (TADs), (Figure 2) [86,87]. The hierarchical structure of inter-TADs contacts is established and mediated by several factors, including architectural proteins, Mediator, tissue specific transcription factors and local RNA transcription [88]. Interactions within TADs correlate with expression levels and variability of chromatin states [89,90]. Transcriptionally non-active, pre-existing interactions are also observed. Jin and colleagues provided evidence, that TNF-responsive gene promoters can be juxtaposed to TNF-responsive regulatory elements prior to stimulation [91]. The authors also suggest that once interactions are established in given cell they might influence target gene expression in cell specific manner.

Epigenetic signatures of specialized TADs have been found to correlate with hormones induced gene regulation [92]. Furthermore, TADs reportedly align with DNA replication domains and were proposed to represent stable units of replication-timing regulation [93]. It is worth noting, that spreading of histone H2AX phosphorylation, induced by ATM kinase in response to DNA damage was detected

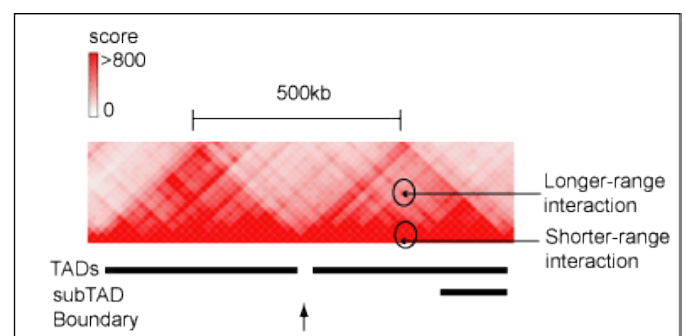


Figure 2. The fragment of Hi-C contacts map for chromosome 8 (<http://www.3dgenome.org>), demonstrating hierarchical structure of TAD. Positions of TAD, sub-TAD, domain boundary as well as long- and shorter-range interactions between genomic regulatory elements are indicated.

along TADs. In contrast to H2AX, ATM kinase has been found locally, on domains borders [94]. Based on this observation it was suggested, that the major role of ATM kinase in DNA damage repair may rely on its ability to modify both local as well as global chromosome organization and chromatin mobility. It is presumed that this occurs with contribution of actin filaments, microtubules and cohesin complexes.

Although sequences within TADs interact preferentially with sites inside the domain, at the edge, inter-domain and inter-chromosomal contacts occur [95]. Borders domains, which separate TADs are enriched in architectural proteins including CTCF and cohesin as well as short interspersed nuclear elements (SINE) and tRNA genes [93]. Their strength can be regulated developmentally, as in case of the border, controlling interactions between HOXD genes and their regulatory elements during mouse limb development [96]. The border strength can also decrease in response to heat shock stress. Heat shock in *Drosophila* was reported to cause an increase of inter-domain and inter-chromosomal interactions between polycomb responsive elements and the subsequent transcriptional silencing of entire TADs domains [97]. Several reports and recent reviews describe the role of local TAD boundary disruption in establishing improper regulatory circuits (between oncogenes and regulatory elements) that can drive neoplastic growth, discussed by [98,99].

As reported by Naumova and colleagues [100], interphase-specific chromosomal organization of TADs is lost in mitotic cells and replaced by a series of cell-invariant, consecutive loops. Dilep and colleagues proposed that TADs and their long-range contacts are reestablished during early G1 cell cycle phase coinciding with the establishment of the replication-timing program [101]. According to Naumova and colleagues higher order chromatin structures that have to form de novo in early G1 do not themselves convey epigenetic memory [100]. Instead, their re-emergence in early G1 is restored by histone marks, DNA methylation, and protein complexes that remain on DNA through mitosis, e.g. at key gene regulatory elements [102] or at TAD boundaries [103]. In addition to polycomb group protein [102], tissue-specific transcription factors [103] a possible role of *Drosophila* CTCF in mitotic bookmarking and maintaining chromatin domains during the cell cycle has been also suggested [104].

In this context it is worth to mention the recent evidence resulted from mapping long-range genomic interactions before and after reprogramming of somatic cells, that have demonstrated that specific long-range contacts are acquired by induced pluripotent cells in cell-type specific-manner during reprogramming [105,106]. According to Gonzales and Ng this indicates existence of topological memory in reprogrammed somatic cells [107].

Long-range contacts were also mapped and analyzed on interphase chromosomes, with close to single regulatory element resolution, by Capture Hi-C (Chi-C), which combines Hi-C methodology with hybridization-based capture of targeted genomic regions. These analyses conducted along with Pol II precipitated interactions (by ChiA-PET), revealed that both active and inactive genes promoters contact each other and form multigenic complexes with correlated expression levels. No bias was detected for active versus non-active promoters [108,109]. According to Rowley and Corces this might indicate the existence of so called "matrix of expression regulation" [98]. Noteworthy, this evidence is also consistent with phenomenon of clustering of co-regulated active and inactive genes observed at nuclear sub-compartments, such as transcriptional factories discussed

by [72], Li and colleagues suggest, that clustering of gene promoters can multiply an effect of any genetic error and/or polymorphism at the single promoter level, depending on the cell specific factors [110]. Their evidence shows that the disease related SNPs are more likely to be found at interacting promoter regions.

Long range interactions of the BRCA bidirectional promoter

To date dynamic long distance interactions between the promoter, introns and terminator regions of the mammalian BRCA1 gene have been reported [111]. The BRCA1 promoter and terminator contacts have been found to suppress estrogen-induced transcription and be potentially linked to dysregulated expression of BRCA1 seen in breast tumors.

Publically available Hi-C contacts maps for chromosome 17, localize the BRCA1/NBR2 promoter region app. 160 kb away from the border of the TADs domain (in esc and IMR90 cells) (Figure S1[A]) (<http://www.3dgenome.org>). Interestingly, this border, demonstrates multiple inter-domain and inter-chromosomal interactions with X chromosomes and autosomes in MCF-7 cells (not shown), (NIH Roadmap Epigenomics Consortium; <http://www.roadmapepigenomics.org/>). Among several tools to analyze or to visualize Hi-C data, reviewed recently by [112,113], Hi-C browser, by Ren lab, provides virtual-4C software, that supplements Hi-C data with DHS linkage and ChIP-Seq evidence (<http://www.3dgenome.org>). The software visualized cis-regulatory potential of SNP rs11655505 (C/T), at the bi-directional BRCA1 promoter (Figure S1[B]) to establish long-range (>10kb) contacts with regulatory elements at the Neighbor of BRCA1 number 1 (NBR1) gene, with the edge of TAD and with domain boundary (Figure S2A[1,2,3]).

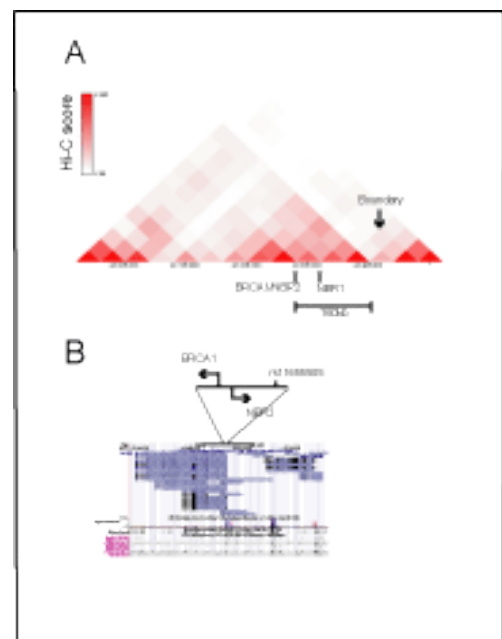


Figure S1. Interactions of the BRCA1 promoter at the Hi-C map for chromosome 17. Position of the genetic variant (rs1165505) at the bi-directional BRCA1 promoter. **Panel A.** Interactions of the BRCA1 bi-directional promoter are mapped app. 160 kb from the border of the TADs domain in esc (<http://genome.ucsc.edu/>). **Panel B.** Polymorphic variant (rs1165505) resides at the bi-directional BRCA1/NBR2 promoter and falls into region of homology to the NBR1 regulatory elements. Position of SNP variant is referred to Ref-seq, DNase hypersensitivity sites (DHS), CTCF binding sites data, (<http://genome.ucsc.edu/>).

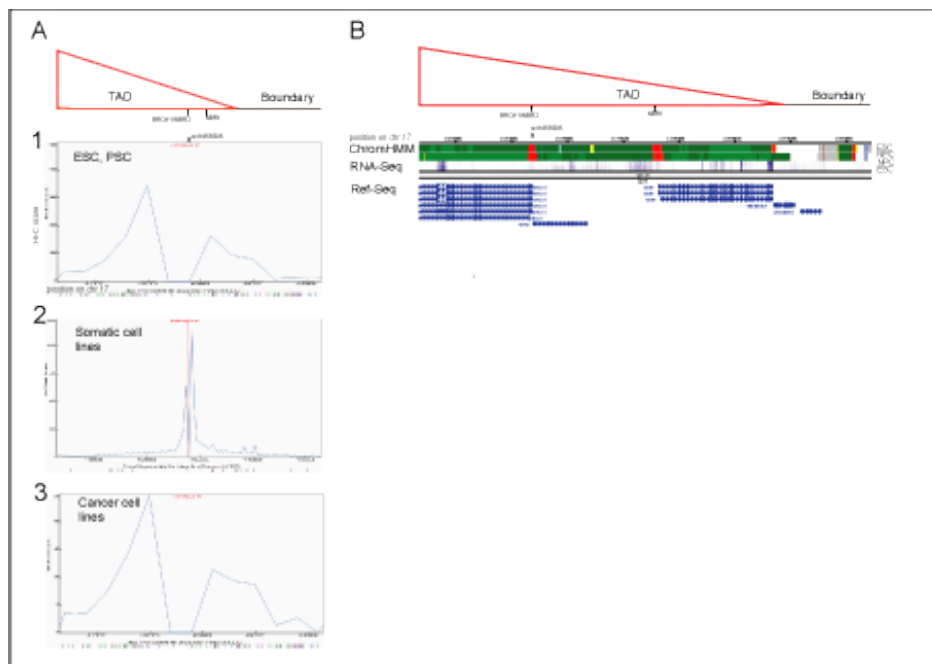


Figure S2

Potential cis-regulatory elements for SNP rs11655505 (C/T), and their chromatin states defined by virtual-4C and chromHMM analysis, respectively.

Panel A

Linear plots of rs11655505 quantified interactions obtained by 4-C virtual analysis (HYPERLINK "<http://www.3dgenome.org/>" <http://www.3dgenome.org/>), which simulates Hi-C data, supplemented with DHS linkage and CHIP-Seq data, aligned with depicted TAD and the boundary (proximal to BRCA1 locus).

A[1]

In embryonic stem cells and progenitor cells (including H1-ESC, H1-MSC, H1-NPC), rs11655505 demonstrates potential to contact the NBR1 gene regulatory element as well as the TADs edge and TADs boundary. The interacting potential of rs11655505 was visualized by Hi-C browser with resolution of 40 kb.

A[2]

In somatic cell lines (including HMEC, HUVEC, NHEK, IMR90, GM12878), rs11655505 shows the highest potential for short-range interactions (<10kb) (within the NBR2 sequences), defined by the software with resolution of 5 kb.

A[3]

In cancer cell lines (including, PANCI, LNCaP, Caki2), patterns of potential long-range interacting elements for rs11655505, (predicted by the software with resolution of 40 kb), are similar to these shown in esc and psc.

Panel B

In esc and psc, potential long-range interacting elements at the BRCA1, NBR2 and NBR1 genes are characterized as the active promoters, by chromHMM.

In these cells, the transcripts of BRCA1, NBR2 and NBR1 are detected by RNA-seq (NIH Roadmap Epigenomics Consortium; <http://www.roadmapepigenomics.org/>).

These long range interactions, of rs11655505 are predicted to occur in embryonic stem cells, progenitor cells (Figure S2A[1]), in somatic (Figure S2A[2]) and cancer cell lines (Figure S2A[3]). Notably, short range interactions (<10kb) with regulatory sequences of NBR2 are predicted to be most frequent for this SNP in somatic cell lines, (Figure S2A[2]).

In H1 embryonic stem cells long-range interacting regulatory elements of the BRCA1, NBR2, NBR1 genes are characterized as active promoters (Figure S2B). Whereas in somatic and in cancer cell lines they are defined as active promoters and/or enhancers (not shown) (ChromHMM; NIH Roadmap Epigenomics Consortium; <http://www.roadmapepigenomics.org/>). Their evolutionary profiles show the presence of subregions with over 98% of homology resulted from segmental duplication. Moreover the BRCA1, NBR1 genes and lncRNA of NBR2 are significantly expressed in H1 embryonic stem cells (RNA-Seq data Roadmap Epigenomics Consortium; <http://www.roadmapepigenomics.org/>) (Figure S2[B]).

As the whole, the publicly available data allow to speculate, that the BRCA1, NBR2 and NBR1 promoters may cluster together and have

correlated transcription level (which might be referred as a putative so called “matrix of expression regulation” in embryonic stem cells, (Figure 3A). Potential interactions between promoters are of special interests because their clustering might multiply the risk of the single genetic variant (SNPs).

Furthermore, the potential contacts of the BRCA1 promoter with TADs edge and/or boundary domain might enable transmission cell-autonomous signals through the inter-domain and inter-chromosomal contacts (Figure 3[A]). One may speculate that these signals could be related to sexual identity and/or parental of origin effects. Notably, sexual identity of adult stem cells with XX karyotypes has recently been reported to have a novel significant role in controlling organ size, plasticity and tumor susceptibility (in *Drosophila* intestine) [114]. The potential interactions might also connect the BRCA1 promoter and its transcriptional machinery, (bearing autoregulatory BRCA1 protein) with ATM kinase (if it is accumulated at the boundary) and modify the risk of BRCA1 mutations.

The establishment of long-range genomic contacts (mediated by numerous nuclear factors) is never a one-sided effect and it may

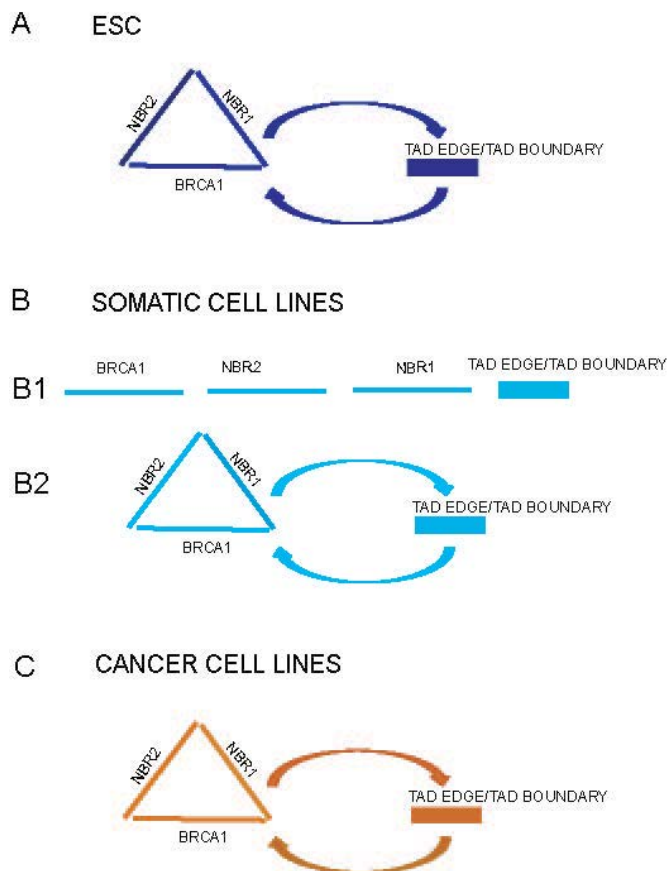


Figure 3. The outline of potential long-range interactions of the BRCA1 promoter (driven by polymorphic variant SNP rs11655505 C>T, as an example). In embryonal stem cells, the BRCA1, NBR2 and NBR1 active promoters may cluster together and/or establish long-range interactions with TADs edge and TADs boundary (Figure 3[A]).

In somatic and cancer cell lines, the active BRCA1, NBR2, NBR1 promoters and/or enhancers potentially interact and contact with TADs edge and TADs boundary (Figure 3B[2], C).

One may speculate, that these potential long-range interactions might increase communication between the BRCA1, NBR2, NBR1 regulatory elements and TADs edge or boundary in cell specific manners, (marked by different colors), (Figure 3A, B[2], C).

The vast majority of cells from somatic cell lines have low potential to make the long-range contacts with regulatory elements of the NBR1 gene and TADs boundary (Figure 3B[1]). Based on visualization of genomic contacts of rs11655505 by 4C-virtual software (<http://www.3dgenome.org>), as well as data from public resources, provided by (NIH Roadmap Epigenomics Consortium; <http://www.roadmapepigenomics.org/>)

influence status of TAD's edge and its border domain. In that case the putative BRCA1 promoter regulatory circuit, (displayed by rs11655505) might have an impact on chromatin status and expression rate of the BRCA1 promoter, many other genes in cis- and in trans- and contribute to the potential epigenetic memory of the region.

In contrast to H1 embryonal stem cells, in somatic and cancer cell lines, the putative long-range interactions may be driven by the BRCA1, NBR2, NBR1 active promoters and/or enhancer regulatory elements (Figure 3B[2], 3C). However, based on virtual 4-C analysis, one may speculate that the vast majority of cells in somatic cell lines does not establish long-range contacts between the BRCA1/NBR2, NBR1 regulatory elements and TADs boundary (Figure 3B[1]).

Conclusions

In each cell BRCA1 mediates molecular processes, which are

critical for genome stability. In mammary gland BRCA1 expression is required for differentiation of breast stem cells and its disturbance may be implicated in aetiology of basal-like breast cancer. Long-range contacts of the bidirectional BRCA1 promoter with the NBR1 regulatory elements, TADs edge and TADs boundary are of interest because they might moderate the BRCA1 promoter communication along chromosome as well as between chromosome and increase BRCA1 expression plasticity in response to genotoxic stress. Once established they could be implicated in maintenance of pluripotency and contribute to potential epigenetic memory of region. They might also multiply the risk of the genetic variants at the BRCA1 promoter due to clustering of promoters (associated with establishment of putative so called "matrix of expression regulation") or to modify the risk of mutations of the BRCA1 protein. Comparative, high resolution mapping and analysis of long-range contacts of the BRCA1 promoter in stem, progenitors, primary somatic and cancer cells, as well as in breast cancer patients, when it becomes attainable, will shed more light on their potential contribution to the risk of breast cancer.

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