**BRCA1—No Matter How You Splice It**

Dan Li¹, Lisa M. Harlan-Williams¹,², Easwari Kumaraswamy¹,³, and Roy A. Jensen¹,²,³,⁴,⁵

**Abstract**

**BRCA1** (breast cancer 1, early onset), a well-known breast cancer susceptibility gene, is a highly alternatively spliced gene. **BRCA1** alternative splicing may serve as an alternative regulatory mechanism for the inactivation of the **BRCA1** gene in both hereditary and sporadic breast cancers, and other **BRCA1**-associated cancers. The alternative transcripts of **BRCA1** can mimic known functions, possess unique functions compared with the full-length **BRCA1** transcript, and in some cases, appear to function in opposition to full-length **BRCA1**. In this review, we will summarize the functional “naturally occurring” alternative splicing transcripts of **BRCA1** and then discuss the latest next-generation sequencing–based detection methods and techniques to detect alternative **BRCA1** splicing patterns and their potential use in cancer diagnosis, prognosis, and therapy.

**Introduction**

**BRCA1** (breast cancer 1, early onset), well known as a breast cancer susceptibility gene, was first mapped to chromosome 17q21 in a hereditary breast cancer study in 1990 (1) and was first cloned in 1994 (2). The **BRCA1** mRNA measures 7.8 kb, with 22 coding and two non-coding exons, producing a 220 kDa protein of 1863 amino acids (2). The **BRCA1** protein contains several important functional domains such as the RING domain (encoded by exons 2–5), and the BRCT domain (encoded by exons 15–23; Fig. 1A; refs. 3–6). **BRCA1** plays a critical role in many important cellular processes, but is best known for its roles in DNA repair, cell proliferation, and transcriptional regulation (7). The inactivation of the **BRCA1** gene has been primarily found in hereditary breast and ovarian cancer cases (2, 8), and in sporadic cases of the basal-like breast cancer molecular subtype (9–12). Inactivation of **BRCA1** has been described in other cancers such as prostate, pancreatic, and colon, but at a very low frequency (13). Germ-line mutations of **BRCA1** account for only 1% to 5% of all breast cancers (2, 14–16), and 40% to 45% of inherited breast cancers (8, 17–19). On the other hand, **BRCA1** inactivating somatic mutations are very rare (20–22), responsible for 0.02% of total breast cancer and 2% of basal-like breast cancer, according to the COSMIC (Catalogue of Somatic Mutations In Cancer) database (cancer.sanger.ac.uk/cosmic/). Therefore, alternative regulatory mechanisms for **BRCA1** dysfunction in patients with breast cancer may play an important role in **BRCA1**-mediated carcinogenesis and as a result this review will focus primarily in this area.

Alternative splicing of precursor mRNA occurs in 92% to 94% of human genes and an average gene has four alternatively spliced transcripts (23–25). Alternative splicing contributes greatly to proteome diversity, tissue/cell type-specific expression patterns, and fine-tuning of gene functions through various regulatory pathways (26–28). Alternative splicing happens both co-translationally and post-translationally. The splicing process is controlled by alternative 5’ or 3’ splice sites, cis-regulatory sequences in the exons or introns (enhancers and silencers), and trans-acting regulatory RNA binding proteins (heterogeneous nuclear ribonucleoprotein hnRNP and tissue-specific factors; ref. 26). The nomenclature of alternative splicing transcripts in this review is based on the previous publications (29–31). The symbol for insertions (▼) or deletions (△) indicates splicing transcripts of a single exon or consecutive exons. The letter ‘p’ or ‘q’ represents a shifting of 5’ss (distal) sites or 3’ss (proximal) sites, respectively.

Because the cloning of the **BRCA1** gene in 1994, alternative splicing transcripts of the **BRCA1** gene have been found in both adult and fetal tissues (2, 32). There is considerable evidence that **BRCA1** is a highly alternatively spliced gene. Recent next-generation sequencing (NGS) studies detected more than 100 **BRCA1** alternative splicing transcripts in human tissues and cells (31, 33, 34). Only a few of these **BRCA1** alternative transcripts have been investigated functionally. The alternative transcripts of **BRCA1** can mimic, function in opposition and in some cases, possess unique functions compared with the full-length **BRCA1** transcript (27). In this review, we will focus primarily on the functional “naturally occurring” alternative splicing transcripts of **BRCA1**, and then discuss recently developed NGS-based detection methods and techniques to detect alternative **BRCA1** splicing patterns and their potential use in cancer diagnosis, prognosis, and therapy. For this review, “naturally occurring” alternative splicing transcripts will be defined as having been identified in a normal cell of any type (and by implication possessing two copies of a normal **BRCA1** gene). It should be noted that there are numerous other, non-naturally occurring **BRCA1** alternative splicing transcripts that arise as a result of specific mutations identified from families with **BRCA1** germ-line mutations (35–39) or from

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**BRCA1 Alternative Splicing Transcripts**

“Naturally occurring” BRCA1 alternative splicing transcripts and their functions are summarized in Fig. 1 and Table 1, respectively.

**BRCA1 Exon 1a, 1b**

The first exon of the BRCA1 gene can be alternatively spliced and can generate two distinct BRCA1 transcripts, exon 1a and exon 1b (Fig. 1B; refs. 42, 43). BRCA1 exon 1a and exon 1b transcripts are produced by the alternative use of dual promoters and distinct transcription start sites, modulated by binding of different trans-acting factors (43). The exon 1a transcript is the major transcript expressed in the mammary gland, whereas the exon 1b transcript is the major transcript expressed in the placenta (30, 42). However, the exon 1b transcript is expressed more in sporadic breast cancer (43). Studies demonstrate that the translation efficiency of the exon 1b transcript is ten times lower than that of the exon 1a transcript. This may contribute to the decrease in BRCA1 protein observed in sporadic breast cancers (43). There is a controversy regarding the existence of an exon 1c transcript using a separate promoter in the BRCA1-IRIS (In-frame Reading of BRCA1 Intron 11 Splice variant) alternative transcript (see the below; refs. 44–46).

**BRCA1α or BRCA1 Δ(11q), BRCA1b or BRCA1 Δ(9, 10, 11q) and BRCA1 Δ(9, 10)**

The BRCA1 Δ(11q), BRCA1 Δ(9, 10, 11q) and BRCA1 Δ(9, 10) transcripts are common, splicing transcripts of BRCA1 mRNA in normal breast cells, and breast and ovarian cancer cell lines (Fig. 1B; refs. 32, 47). However, at the protein level, the presence of these three alternative transcripts was only proved for the Δ(11q) and Δ(9,10,11q) transcripts, named BRCA1α and BRCA1b, respectively (29, 32, 48–50). These two BRCA1 splicing transcripts were referred to by different names in the previous literature. BRCA1α was referred to as BRCA1 Δ11b (51), BRCA1a (47, 48), BRCA1 D11q (52), and BRCA1 Δ(11q) (32). BRCA1b was referred to as BRCA1 Δ(9,10) (47, 48) and BRCA1 Δ(9,10,11q) (32). BRCA1a (110 kDa, p110) and BRCA1b (100 kDa, p100) both have the majority of exon 11 deleted, compared with full-length BRCA1 (220 kDa, p220; refs. 49, 53, 54). BRCA1b has two more exons deleted (exon 9, 10) compared with BRCA1a (49, 53, 54).

Because of the deletion of the majority of exon 11, BRCA1a and BRCA1b lack specific binding domains for selected proteins, including RB, p53, MYC, RAD50, TUBG (γ-tubulin) and angiopoietin-1 (Fig. 1A; ref. 53). Other RB- and p53-binding domains
Table 1. Functional naturally occurring BRCA1 alternative splicing transcripts

<table>
<thead>
<tr>
<th>Splicing transcripts</th>
<th>Alternative names</th>
<th>Molecular weight (kDa)</th>
<th>Notes</th>
<th>Disease association</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 exon 1a (42, 43)</td>
<td></td>
<td>220 (1863 a.a.)</td>
<td>• The exon 1a transcript-mainly expressed in mammary gland (30, 42).</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>BRCA1 exon 1b (42, 43)</td>
<td></td>
<td></td>
<td>• The exon 1b transcript-mainly expressed in placenta and sporadic breast cancer (42, 43).</td>
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<tr>
<td>BRCA1 exon 1c? (44–46)</td>
<td></td>
<td></td>
<td>• Translation efficiency of the exon 1b transcript is much lower than that of the exon 1a (44).</td>
<td></td>
</tr>
<tr>
<td>BRCA1a (49, 50, 54, 58, 136)</td>
<td>BRCA1 A(19q) (32)</td>
<td>110 (760 a.a.)</td>
<td>• Functions as a growth/tumor suppressor of steroid hormone-independent human breast, ovarian, and prostate cancer cells (53).</td>
<td>Breast cancer</td>
</tr>
<tr>
<td></td>
<td>BRCA1 A1b1 (51)</td>
<td></td>
<td>• The RB pathway, rather than the p53 status, can affect the ability of BRCA1a to inhibit tumor growth of cancer cells (53).</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td></td>
<td>BRCA1 D11q (52)</td>
<td></td>
<td>• Transcriptional activity of BRCA1a is overlapping, but unique compared with the full-length BRCA1 (60).</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td></td>
<td>p150 (44)</td>
<td></td>
<td>• Nuclear BRCA1a promotes cell apoptosis, whereas cytoplasmic BRCA1a retention promotes clonogenicity of MCF7 breast cancer cells (9, 55, 57).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRCA1b (49)</td>
<td>100 (719 a.a.)</td>
<td>• Mitochondria forms of BRCA1a and BRCA1b possess antiproliferative activity in breast cancer cells (58).</td>
<td>Breast cancer</td>
</tr>
<tr>
<td></td>
<td>BRCA1 A(9, 10, 11q) (32)</td>
<td></td>
<td>• The expression of BRCA1a and BRCA1b varies among different cancer cells and during different stages of the cell cycle (52, 51, 55).</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td></td>
<td>BRCA1a_{9,9.10} (47, 48, 60)</td>
<td></td>
<td></td>
<td>Leukemia</td>
</tr>
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<td></td>
<td>p100 (49)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BRCA1-IRIS* (44, 61)</td>
<td>p150 (44)</td>
<td>150 (1399 a.a.)</td>
<td>• Highly expressed in some breast and ovarian cancer cell lines (44, 63, 64).</td>
<td>Breast cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Exclusively localized in the nuclear chromatin (44).</td>
<td>Ovarian cancer</td>
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<td></td>
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<td>• Promotes the formation of aggressive and invasive breast cancers (65–67).</td>
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<td>• Overexpression can also promote paclitaxel resistance in TNBC breast cancers (71).</td>
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*IRIS, in-frame reading of BRCA1 intron 11 splice variant).

Located at the BRCA1 C-terminus (exon 16–24) remain intact (Fig. 1A). BRCA1a can still function as a growth/tumor suppressor of steroid hormone-independent human breast, ovarian, and prostate cancer cells. In this variant, the RB pathway, rather than the p53 status, appears to determine the ability of BRCA1a to inhibit tumor growth (53).

Furthermore, BRCA1a and BRCA1b lack the nuclear localization signal located in exon 11 (Fig. 1A; ref. 51). Thus, BRCA1a and BRCA1b are mainly cytoplasmic (55). However, there is still a small portion of BRCA1a and BRCA1b localized in the nucleus, transported by an alternative mechanism (48, 49, 51, 56). The function of BRCA1a seems to depend on its subcellular localization (19). Nuclear BRCA1a promotes cell apoptosis, whereas cytoplasmic BRCA1a promotes clonogenicity of MCF7 breast cancer cells (19, 55, 57). In addition to nuclear localization and cytoplasmic localization, one study reported that BRCA1a, BRCA1b, and BRCA1b also localize to the mitochondria when overexpressed in MCF-7 cells and other cell lines (58, 59). The mitochondrial BRCA1a and BRCA1b possess antiproliferative activity in breast cancer cells (58).

Transcriptional activity of BRCA1a and full-length BRCA1 was evaluated in a microarray study of 1176 genes (60). BRCA1a has overlapping, but unique transcriptional activity compared with full-length BRCA1, with variations from the full-length transcript across selected gene sets (60). Like full-length BRCA1, the transcriptional activation of BRCA1a and BRCA1b depends on p53 (48). Interestingly, more-than-additive transcriptional activation of the p21 promoter was observed for the co-transfection of full-length BRCA1 with BRCA1a or BRCA1b (48). In addition, the expression levels of BRCA1a and BRCA1b alternative splicing transcripts vary among different cell types (breast cancer, ovarian cancer and leukemia cells; refs. 32, 51) and during different stages of the cell cycle (55).

**BRCA1-IRIS**

In 2004, the BRCA1-IRIS (in-frame reading of BRCA1 intron 11 splice variant) was identified by cDNA cloning and was found to be predominantly expressed in selected human tissues (fetal skeletal muscles and adult leukocytes) and cancer cell lines (Hs578T and HCC1937; Fig. 1B; ref. 44). BRCA1-IRIS mRNA contains an uninterrupted open reading frame from codon 1 in exon 2 to the end of exon 11 of full-length BRCA1. It then continues in-frame for 34 more triplets into intron 11 and then terminates. BRCA1-IRIS encodes a 1,399-amino-acid protein (p150) (44, 61). Therefore, BRCA1-IRIS protein shares the same 1365 amino acids at the N-terminus with full-length BRCA1, but lacks the C-terminal (BRCT) domain of the full-length BRCA1. Although BRCA1-IRIS retains the RING finger located at the N-terminus, it does not interact with the BRCA1 associated RING domain (BARD1). It suggests that the conformation of RING finger of BRCA1-IRIS differs from that of the full-length BRCA1, even though their primary sequences are identical (44).
The BRCA1-IRIS transcript is expressed at a level five times greater than that of full-length BRCA1 mRNA in adult peripheral white blood cells. It is also expressed in the adult spleen, in which the full-length BRCA1 is absent. Unlike the full-length BRCA1, BRCA1-IRIS mRNA and protein are exclusively localized in nuclear chromatin. The BRCA1-IRIS promoter is active in the G0 phase in some cell lines and the transcript can be detected in both the G0 and S phases during the cell cycle (44). Interestingly, BRCA1-IRIS overexpression is associated with the absence of full-length BRCA1 expression (44, 62). BRCA1-IRIS is highly expressed in some breast and ovarian cancer cell lines, especially in the HCC1937 cell line, which does not express any full-length BRCA1 transcript because of BRCA1 genetic mutations (44, 63, 64). One study demonstrated that the loss of full-length BRCA1 can trigger BRCA1-IRIS overexpression by increasing BRCA1-IRIS mRNA stability (65).

Unlike the full-length BRCA1, BRCA1-IRIS promotes the formation of aggressive and invasive breast cancers (65–67). In patients with triple-negative breast cancer (TNBC), high expression of BRCA1-IRIS correlates with aggressive clinical behavior of breast cancer, including lymph node metastasis, local recurrence, distant metastasis, and decreased survival (66, 67). Both in vitro cell studies and in vivo xenograft mouse models demonstrate that BRCA1-IRIS overexpression promotes TNBC tumor formation, invasion, and migration (62, 66–71). The proposed mechanism is that BRCA1-IRIS may increase DNA replication and cell proliferation (44), induce transcription of oncogenes cyclin D1 and EGFR (69, 71, 72), suppress full-length BRCA1 expression (62), activate WIP1 phosphatase (73, 74), enhance EMT (epithelial-mesenchymal transition), upregulate transcription factors associated with stemness, and activate the tumor-initiating cells (TIC) phenotype in TNBC cells (62). BRCA1-IRIS overexpression can also promote paclitaxel resistance in TNBC breast cancers, partly by activating an autocrine signaling loop of EGF/EGFR-ErbB2 and NRG1/ErbB2-ErbB3 (71).

Studies in ovarian cancer also show that BRCA1-IRIS acts as a tumor promoter (61, 70) and promotes metastasis and drug resistance (68). BRCA1-IRIS overexpression can also trigger survival expression and lead to cisplatin resistance in ovarian cancer cell lines (75). Thus, BRCA1-IRIS may have potential as a biomarker for diagnosis and prognosis in breast and ovarian cancer, and a predictor for chemotherapy response (68).

**Comprehensive BRCA1 Alternative Splicing Study**

Microarrays, such as splice junction arrays, exon arrays, cDNA arrays, and tiled genomic arrays, have been widely used for the large-scale analysis of splicing with various advantages and disadvantages (76, 77). Recently, more systematic and comprehensive studies of “naturally occurring” BRCA1 alternative splicing patterns have been conducted. In 2014, the ENIGMA (www.enigmaconsortium.org) consortium detected BRCA1 alternative splicing in RNA sources from 48 healthy blood and one healthy breast tissue (29). In this study, 63 BRCA1 alternative splicing transcripts were characterized including 35 novel transcripts. Ten alternative splicing transcripts among those 63 transcripts were predominant, according to their expression relative to the full-length transcript: ΔA1q, Δ5, Δ5q, Δ8p, Δ9, Δ(9,10), Δ9_11, Δ11c, Δ13p and Δ14p (29). In 2015, a comprehensive study of BRCA1 alternative splicing transcripts was conducted in 70 breast tumors from patients and four healthy breast tissues (30). In this study, 54 out of 63 BRCA1 splicing transcripts identified in the blood (29) were also found in breast tissue samples (30). Five BRCA1 transcripts are expressed at much higher levels in breast tumors than in healthy blood controls: Δ5q, Δ13, Δ9, Δ5 and ▼14A. In both studies, the alternative mRNA transcripts were discovered by RT-PCR, exon scanning, cDNA cloning, and sequencing. These traditional methods can only target partial transcript sequences of BRCA1.

More recently, high throughput RNA sequencing (RNA-Seq) has been used to detect novel BRCA1 alternative transcripts in the whole transcriptome (31, 33, 34, 78, 79). A high-throughput targeted RNA-Seq was applied to investigate the global splicing pattern of BRCA1 and 10 other hereditary breast and ovarian cancer (HBOC)–related genes in patients with HBOC (33). Five new alternative splicing events were identified in BRCA1 in this study. Furthermore, to improve the analysis of exon–exon junctions in RNA-Seq data, a nanopore sequencing method was developed. Nanopore sequencing provides long-read sequence data to fully characterize the exon connectivity in the transcript (80). A study using the nanopore sequencing method identified 32 complete BRCA1 isoforms including 20 novel isoforms in a human healthy lymphoblastoid cell line (34). In addition, a more sensitive and rapid method of multiplex PCR and NGS has been developed. Multiplex PCR can amplify all theoretically possible exon–exon junctions, followed by NGS to characterize PCR products (31). Using this method, there are 94 BRCA1 alternative splicing transcripts observed in human leukocytes, normal mammary, adipose tissues, and stable cell lines. Interestingly, there is only a minor difference between human leukocytes and breast tissues (29–31, 33). The similarity of BRCA1 alternative transcripts pattern in the blood and in the breast tissues supports the use of blood-based splicing evaluation assays in further studies. In addition, the vast majority of BRCA1 alternative splicing transcripts identified have no known function, but in some cases, appear to serve as non-coding or nonsense transcripts.

**New Techniques and Perspectives for Studying Regulatory Mechanisms of BRCA1 Alternative Splicing**

Recently, new techniques have been developed to detect the expression level of a single splicing transcript. Compared with traditional qPCR, droplet digital PCR was developed to provide a more sensitive, specific, and precise quantification of alternative splicing transcripts (81, 82). In addition, a spectroscopic strategy can be utilized for quantitative imaging of alternative splicing transcripts. In this method, the spatial, temporal, and quantitative expression of a single BRCA1 splicing transcript can be monitored at single copy resolution in live cells, which can potentially enhance the understanding and significance (function) of alternative mRNA splicing (83, 84). Furthermore, saturation genome editing has been used to accurately detect BRCA1 variants, including BRCA1 alternative splicing variants (3). In addition to detecting the mRNA profiles, multiple approaches have emerged to identify RNA-binding proteins (RBP)–RNA interactions (77, 85–91). Understanding of RBPs–RNA interaction of BRCA1 could help identify the regulatory mechanism of BRCA1 mRNA alternative splicing.
Modification of BRCA1 Splicing in Treatment Strategy

Patients carrying breast cancer with BRCA1 deficiency are more sensitive to treatment with a combination of PARP inhibitor and DNA-damaging agents (92–94). A recent study tried to mimic BRCA1 deficiency to improve PARP inhibitor sensitivity in cell lines. It showed that the full-length BRCA1 transcript can be modified into the BRCA1 Δ11 transcript through Splice-Switching Oligonucleotide (SSO). SSO anneals to pre-mRNA sequences and simulates exon 11 skipping of endogenous BRCA1 pre-mRNA. Wild-type BRCA1-expressing cells when transfected by SSO, become more susceptible to PARP inhibitor treatment (95).

Alternatively, aberrant splicing can be corrected by introducing hybrid protein nucleic acid (PNA)-peptide oligomers, called ESSENCE (exon-specific splicing enhancement by small chimeric effectors), to mimic the functions of splicing factor Serine/arginine-Rich (SR) proteins (96). ESSENCE can specifically restore the exon 18 skipping of BRCA1 E1694X to the wild-type full-length BRCA1 splicing in in vitro splicing assays (97). However, the efficacy of synthetic effectors needs to be further evaluated in vivo before therapeutic application (97, 98).

Potentially, BRCA1-IRIS inactivation may also be a therapeutic strategy in aggressive breast tumors (62, 66). Studies in TNBCs showed that an IRIS-inhibitory peptide can inhibit TNBC progression (62, 71) and sensitize TNBC cells to low paclitaxel concentrations (71) both in vitro and in vivo. In addition, in vitro ovarian cancer cell lines and an in vivo mouse xenograft study demonstrate that use of a BRCA1-IRIS inhibitory peptide significantly decreased ovarian tumor growth and sensitized ovarian tumors to lower cisplatin concentrations (68).

Summary and Future Perspectives

A balanced ratio of BRCA1 alternative splicing transcripts seems to be important for the normal physiological function of BRCA1. Dysregulation of BRCA1 alternative splicing transcripts may contribute to the pathogenesis of breast and/or ovarian cancer, even in non-carriers of BRCA1 germ-line mutations. Comprehensive studies of BRCA1 alternative splicing patterns demonstrate that the peripheral blood has a similar splicing pattern to normal breast tissue, although selected BRCA1 alternative splicing transcripts may have tissue/cell-type specificity (29, 30, 33). Although many BRCA1 alternative splicing transcripts have been identified, the vast majority have no known function, and in most cases, there are no currently available antibodies capable of recognizing and distinguishing the multitude of translated BRCA1 protein isoforms. The alternative transcripts that lack coding sequences (99) or induce the nonsense-mediated mRNA decay (NMD) pathway (29, 100), may serve as stochastic noise in the transcription and splicing process (101). These issues pose a major challenge to understanding the full impact of the BRCA1 gene, which undoubtedly is much more complex than our current assessment largely based on the functions attributable to the full-length transcript. A comprehensive and integrated understanding of BRCA1 alternative splicing and characterization of the resulting protein isoforms will be critical to fully leverage BRCA1 as a potential biomarker or therapeutic approach.

As alluded to earlier, gene mutations can produce non-naturally occurring alternative transcripts. BRCA1 synonymous mutations may influence the genetic elements (sequences), which can alter the splicing pattern, such as the 5′-UTR and 3′-UTR, introns, splice sites, and cis-acting splicing regulatory elements/motifs (splicing enhancers and splicing silencers; refs. 102–113). In addition, many BRCA1 germ-line mutations have been previously identified as variants of unknown significance (VUS; refs. 17, 114), but were subsequently found to target critical functional regions of the gene. A considerable number of these VUS were found to interfere with the RNA splicing process. These VUS can produce new splicing transcripts or affect the ratio of naturally occurring alternative splicing transcripts of the BRCA1 gene (110, 115–126).

BRCA1, as a multi-functional gene, is involved in numerous physiological functions and signaling pathways. Recently, alternative splicing transcripts have been found in “BRCA1-associated (related)” genes, such as SMARCA4 (BRG1), causing nuclear-cytoplasmic shuttling abnormalities (127), and BARD1 (128, 129). In the FANC-BRCA DNA repair pathway, AKT1 (130), ELK-1 (131), RBCK1 (132), SRSF3 (133, 134), and a number of other genes are prone to significant R-loop formation including p53, BRCA2, KRAS (135, 136). Furthermore, mRNA splicing from precursor mRNAs to mature mRNAs is a highly dynamic and integrated process with crosstalk and interaction with transcription, post-transcriptional modification, and chromatin modification. Therefore, a multi-dimensional understanding among different regulatory layers needs to be considered, as well as the spatial, physical, and temporal availability of all regulatory components in the various cell organelles, subcellular structure, and compartments (27). In addition, alternative splicing can have an important impact on other gene regulatory layers such as mRNA turnover and protein translation (27). In future studies, it will be important to decipher potential alternative splicing mechanisms, and to predict alternative splicing patterns in specific physiologic or pathologic conditions. Moreover, it will be equally important to define the interaction between chromatin and transcription/post-transcriptional-associated components/processes, and the regulatory mechanisms influencing alternative splicing outcomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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