Review

Dualistic Role of BARD1 in Cancer

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Abstract: BRCA1 Associated RING Domain 1 (BARD1) encodes a protein which interacts with the N-terminal region of BRCA1 in vivo and in vitro. The full length (FL) BARD1 mRNA includes 11 exons and encodes a protein comprising of six domains (N-terminal RING-finger domain, three Ankyrin repeats and two C-terminal BRCT domains) with different functions. Emerging data suggest that BARD1 can have both tumor-suppressor gene and oncogene functions in tumor initiation and progression. Indeed, whereas FL BARD1 protein acts as tumor-suppressor with and without BRCA1 interactions, aberrant splice variants of BARD1 have been detected in various cancers and have been shown to play an oncogenic role. Further evidence for a dualistic role came with the identification of BARD1 as a neuroblastoma predisposition gene in our genome wide association study which has demonstrated that single nucleotide polymorphisms in BARD1 can correlate with risk or can protect against cancer based on their association with the expression of FL and splice variants of BARD1. This review is an overview of how BARD1 functions in tumorigenesis with opposite effects in various types of cancer.

Keywords: BARD1; tumor suppressor; genetic variants; cancer predisposition

1. Introduction

In 1996, Wu et al. in effort to understand the function of BRCA1 they used a yeast two-hybrid screen to identify proteins that associate with it in vivo [1]. By this analysis the BRCA1-associated RING domain 1 (BARD1) protein was discovered as a binding partner of BRCA1. BARD1 protein is encoded by sequences on chromosome 2q35 and forms a functional heterodimer with BRCA1 through the binding of their RING-finger domains which functions as tumor-suppressor in breast and ovarian cancer [1–4]. The full length (FL) BARD1 mRNA includes 11 exons and encodes a protein comprising of one N-terminal RING-finger domain, three Ankyrin repeats (ANK) domains and two C-terminal BRCT domains (Figure 1). The recognizable protein motifs of BARD1 are well conserved in mouse [5,6], Xenopus laevis [7], Caenorhabditis elegans [8] and Arabidopsis thaliana [9], including the RING domain, the three tandem Ankyrin repeats and, to a lesser extent, the two BRCT domains. This complexity of structure indicates that BARD1 could have multiple functions.

Conditional inactivation of Bard1 in mice induces mammary carcinomas that are indistinguishable from carcinomas induced by conditional knock-out of Brca1, which establishes BARD1 itself as a tumor suppressor [10]. The knock-out of Brca1 and Brca2 genes in mice led to embryonic lethality. Similarly, homozygous disruption of Bard1 in mice results in lethality between embryonic days E7.5 and E8.5, at time when Bard1 but not Brca1 expression is maximal [5,11]. The phenotype of Bard1 knock-out mice demonstrated that Bard1 is essential for cell viability and maintenance of genome integrity and embryos lethality only after eight days of development could mean that Bard1 deficiency is deleterious to the cells. This hypothesis is supported by the finding that BARD1 mutations are associated with
few cases of non-BRCA1/BRCA2-related sporadic breast and ovarian tumors and account for only a small fraction of cases of familial breast cancer overall [12–16]. Interesting to note, BRCA1 mutations do not immediately result in malignant phenotype but have cumulative effect that is possibly caused by incorrect stoichiometry with interacting proteins [17].

The BARD1-BRCA1 heterodimer has ubiquitin ligase activity that targets proteins involved in cell-cycle regulation, DNA repair, hormone signaling and modulating chromatin structure [18,19]. Several reports show that BARD1 has an additional BRCA1-independent tumor suppressor function in cancer that is antagonized by the expression of BARD1 isoforms. Briefly, the expression of FL BARD1 (tumor suppressor role) is required for genomic stability and cell cycle control; in cancer initiation and progression the expression of BARD1 isoforms (oncogenes) antagonize FL BARD1 functions and permit uncontrolled proliferation (Figure 1B) [5,20–23]. In the following review, we have focused on the genetic and molecular mechanisms of the dualistic role of BARD1 as oncogene and tumor-suppressor in cancer.

![Figure 1. Structure of BRCA1-associated RING domain 1 (BARD1) and spliced isoforms. (A) Full-length (FL) BARD1 exon structure is aligned with spliced BARD1 isoforms below and protein structure above. The protein domains are reported at top of the figure. Splice variants are named with Greek letters (left). Presumed protein coding exons are shown in blue colors; non-coding exons are shown in white (β, κ, γ, η); asterisk shows alternative open reading frames (β, γ and η). Amino acid (aa) number is reported for FL BARD1 and BARD1 isoforms; (B) Model for dual role of BARD1 in cancer. In normal cells BARD1 isoforms (β, δ, ω isoforms, discussed in “Biological Functions of BARD1 as Oncogene” paragraph) are not expressed; in cancer cells, full-length BARD1 (FL) expression (tumor suppressor role of BARD1) decreases and BARD1 isoforms expression (oncogenic role of BARD1) increases. Figure 1 has been modified from Irmgard Irminger-Finger et al. [3].](image-url)
2. Rare and Common Cancer-Associated Genetic Variants of BARD1

2.1. Rare Predisposing Variants of BARD1 in Cancer

Mutations in the BRCA1 and BRCA2 genes are the most common causes of hereditary breast and ovarian cancer and are associated with a lifetime risk of breast cancer of 50–85% and of ovarian cancer of 15–40%. It is now apparent that mutations of several other genes, such as BARD1, PALB2 (Partner And Localizer Of BRCA2) and BRIP1 (BRCA1 Interacting Protein C-Terminal Helicase 1) [24], contribute to familial breast cancer. BARD1 mutations are expected to account for additional cases of non-BRCA1/2 inherited breast cancer and have been reported in non-BRCA mutated breast cancer families [25–28]. A recent work has suggested BARD1 as cancer-associated gene in ovarian cancer by a case-control association analysis between 1915 patients and Exome Sequencing Project (ESP, http://varianttools.sourceforge.net/Annotation/EVS) and Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org) controls [24]. The authors report a mutation frequency for BARD1 of 0.2% and Odd Ratio of 4.2 (95% confidence interval: 1.4–12.5). Similar results have been presented by Couch et al. from multigene panel-based clinical testing for pathogenic variants in inherited cancer genes among patients with breast cancer [29]. The case-control association analysis between 38,326 white patients with breast cancer and 26,911 ExAC controls demonstrated an association between pathogenic rare variants in BARD1 with a moderate risk value (Odd Ratio, 2.16; 95% confidence interval: 1.31–3.63) and a mutation frequency of 0.18% [29]. Thus, most of the published data are consistent with BARD1 involvement in breast and ovarian cancers susceptibility [12–16,25–29]. Indeed, BARD1 is now included on clinical gene panels for testing for susceptibility to these two tumors. However, no recurrent hotspot variant has been identified so far.

Beyond single nucleotide variants, other types of risk mutations have been found in BARD1 such as splicing mutations and large deletion. Interestingly, Ratajska et al. identified 16 BARD1 mutations in BRCA1/2-negative high-risk breast and/or ovarian cancer patients from Poland [30]. Among these mutations, a splice mutation (c.1315-2A > G) resulted in exon 5 skipping and a silent change (c.1977A > G) which altered several exonic splicing enhancer motifs in exon 10 and resulted in a transcript lacking exons 2–9 [30]. In a recent study, three BARD1 mutations were identified that alter splicing leading to skipping of exons 5, 8 and 2–9, respectively [31].

The Table 1 shows the list of mutations (n = 79) defined as “Pathogenic” and “Likely Pathogenic” in ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). Most of mutations are loss-function due to deletion, nonsense or frame shift mutations and are associated with susceptibility to breast cancer (Table 1 and Figure 2). Only one missense mutation is reported even if recent literature reports diverse potential pathogenic missense mutations in BARD1 [24,29]. Moreover, others and we have demonstrated that BARD1 is enriched in rare, potentially pathogenic, germline variants also in neuroblastoma patients [32,33]. Particularly, the nonsense variant (rs587781948; exon 2), included in ClinVar, has been found in two patients in these two different gene-sequence projects. Based on these observations a curated update of BARD1 mutations in ClinVar database is needed. We also expect that massive sequencing of BARD1 in breast, ovarian cancers, neuroblastoma and other tumors will increase the number of rare pathogenic missense mutations to be inserted into the ClinVar database as “Pathogenic”. However, these data strongly support the role of tumor-suppressor of BARD1 in different cancers.
2. Rare and Common Cancer-Associated Genetic Variants of BARD1

2.1. Rare Predisposing Variants of BARD1 in Cancer

Different copy number variants of BARD1 locus have been found associated with congenital conditions (hypospadias and congenital heart defects: coarctation of aorta and tetralogy of fallot) and developmental phenotypes (Table 1) [34]. Neuroblastoma, tetralogy of fallot and coarctation of aorta are related to tissues that originate from neural crest cells. Moreover, literature data report cases of patients with coexisting neuroblastoma and congenital heart defects [35]. In 2004 George et al. demonstrated that congenital heart defects are more common in neuroblastoma patients than in a control group of children with another type of cancer [36]. Another study has demonstrated that depleting frog embryos of BARD1 leads to defective developmental phenotypes (for instance: malformed neural tube and eye structures) [7]. Together, these evidences indicate that BARD1 might play a role in early organogenesis; however, additional studies are needed to demonstrate this hypothesis.

Although variants in protein-coding regions have received the most attention, numerous studies have noted the importance of non-coding variants in cancer. A sequencing of 20 complete genes, including noncoding and flanking sequences, in hereditary breast and ovarian cancer patients \( n = 287 \) identified a single nucleotide variants in 5' UTR (c.-53G > T; rs143914387) of BARD1 predicted to alter the mRNA structure [37]. Further complete gene sequencing or whole genome sequencing projects are warranted to investigate the contribution of rare non-coding variants of BARD1 in conferring cancer risk.

![Figure 2. BARD1 germline coding mutations. The protein domains RING (green), Ankyrin (ANK, red), BRCA1 carboxy-terminal (BRCT, orange) are indicated. Coding mutations of BARD1 defined as “Pathogenic” and “Likely Pathogenic” in ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) are shown. The red arrows indicate the mutations categorized as “Likely Pathogenic”, the other mutations without the arrow are categorized as “Pathogenic”.](image-url)
| Variation ID | GRCh37 Location | Ref. | Alt | Mutation Type | Protein Change | dbSNP | Frequency in ExAC Database | Condition(s) | Clinical Significance (Last Reviewed) |
|-------------|-----------------|------|-----|---------------|---------------|-------|---------------------------|--------------|---------------------------------------|
| 237823      | -               | -    |     | deletion      | -             | -     | -                         | Familial cancer of breast | Pathogenic (Last reviewed: 10 December 2015) |
| 230523      | 215593433–215593434 | TG   | -   | frameshift deletion | V767fs         | rs790413473 | 0.0006 | Familial cancer of breast | Conflicting interpretations of pathogenicity (Last reviewed: 18 August 2016) |
| 185366      | 215593466       | G    | A   | nonsense      | W756 *         | rs796202138 | - | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 20 June 2014) |
| 187542      | 215593385–215593386 | CA   | -   | frameshift deletion | I717fs         | rs786203811 | - | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 10 December 2014) |
| 422806      | 215593671       | A    | AA  | frameshift duplication | D689fs         | -     | -                         | not provided | Likely pathogenic (Last reviewed: 29 November 2016) |
| 265365      | 215593734       | A    | C   | splice acceptor | D689fs         | rs876658260 | - | Hereditary cancer-predisposing syndrome | Likely pathogenic (Last reviewed: 17 November 2015) |
| 229902      | 215593734       | A    | T   | splice acceptor | D689fs         | rs876658260 | - | Hereditary cancer-predisposing syndrome | Likely pathogenic (Last reviewed: 17 November 2015) |
| 182051      | 215595140       | C    | T   | nonsense      | Q666 *         | rs730881422 | - | Familial cancer of breast | Pathogenic/Likely pathogenic (Last reviewed: 13 January 2017) |
| 187445      | 215595166       | C    | -   | frameshift deletion | P657fs         | rs786203739 | - | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 3 March 2016) |
| 439952      | 215595182–215595201 | frameshift duplication | Q652fs         | rs886039589 | - | Familial cancer of breast | Pathogenic (Last reviewed: 13 April 2017) |
| 265510      | 215595182–215595201 | frameshift deletion | C645fs         | rs876658260 | - | Hereditary cancer-predisposing syndrome | Likely pathogenic (Last reviewed: 3 December 2015) |
| 127725      | 215595182–215595201 | frameshift duplication | Q652fs         | rs587780024 | - | Familial cancer of breast | Pathogenic/Likely pathogenic (Last reviewed: 13 April 2017) |
| 142499      | 215595203–215595204 | frameshift deletion | C645fs         | rs587782534 | - | Hereditary cancer-predisposing syndrome | Pathogenic/Likely pathogenic (Last reviewed: 20 November 2015) |
| 141702      | 215595215       | C    | T   | nonsense      | R641 *         | rs587781948 | 0.0001 | Familial cancer of breast | Pathogenic (Last reviewed: 4 October 2016) |
| 232108      | 215595234       | A    | -   | splice acceptor | D689fs         | rs786659560 | - | Hereditary cancer-predisposing syndrome | Likely pathogenic (Last reviewed: 29 May 2015) |
| 219763      | 215595234       | A    | T   | splice acceptor | D689fs         | rs864622239 | - | Hereditary cancer-predisposing syndrome | Likely pathogenic (Last reviewed: 8 August 2015) |
| 233167      | 215609970       | G    | T   | splice donor  | D689fs         | rs876660237 | - | Hereditary cancer-predisposing syndrome | Likely pathogenic (Last reviewed: 6 August 2015) |
| Variation ID | GRCh37 Location | Ref. | Alt | Mutation Type | Protein Change | dbSNP | Frequency in ExAC Database | Condition(s) | Clinical Significance (Last Reviewed) |
|-------------|-----------------|------|-----|---------------|----------------|-------|----------------------------|--------------|----------------------------------------|
| 232127      | 215609822       | T    | -   | frameshift deletion | L625fs         | rs876659572 | -                          | not provided | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 26 July 2016) |
| 143017      | 215609876-215609877 | AT   | -   | frameshift deletion | H606fs         | rs587782897 | -                          | not provided | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 8 June 2016) |
| 245803      | 215609884       | G    | A   | splice acceptor | -              | rs879253952 | -                          | not provided | Pathogenic (Last reviewed: 9 June 2015) |
| 246449      | 215609885-215609893 | -    | -   | splice acceptor | -              | rs879254264 | -                          | not provided | Likely pathogenic (Last reviewed: 7 March 2016) |
| 232643      | 215610445       | G    | A   | splice donor | -              | rs876659894 | -                          | Hereditary cancer-predisposing syndrome | Likely pathogenic (Last reviewed: 30 June 2015) |
| 127720      | 215610356       | C    | T   | nonsense     | Q664*           | rs587780021 | 0.00005                    | Familial cancer of breast | not provided | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 3 January 2017) |
| 406791      | 215617170       | G    | C   | splice donor | -              | rs106051310 | -                          | Familial cancer of breast | Pathogenic (Last reviewed: 2 August 2016) |
| 141384      | 215617196       | C    | G   | nonsense     | S551 *          | rs587781707 | -                          | Familial cancer of breast | not provided | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 29 December 2016) |
| 230372      | 215617209       | G    | T   | nonsense     | E347 *          | rs876658429 | -                          | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 28 January 2015) |
| 406768      | 215617214-215617248 | frameshift indel | T534fs | rs1064792931 | -                          | Familial cancer of breast | Pathogenic (Last reviewed: 22 September 2016) |
| 379750      | 215617226       | C    | A   | nonsense     | S541 *          | rs777932955 | -                          | not provided | Pathogenic (Last reviewed: 14 May 2015) |
| 406776      | 215632275       | -    | A   | frameshift duplication | D500fs         | -              | -                          | Familial cancer of breast | Pathogenic (Last reviewed: 13 August 2016) |
| 421820      | 215633955       | -    | G   | splice donor duplication | -              | -              | -                          | not provided | Pathogenic (Last reviewed: 4 August 2016) |
| 233414      | 215634002       | -    | A   | frameshift duplication | N450fs         | rs876660390 | -                          | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 17 August 2015) |
| 496748      | 215634026       | C    | -   | frameshift deletion | P442fs         | rs106051287 | -                          | Familial cancer of breast | not provided | Pathogenic (Likely pathogenic (Last reviewed: 20 September 2016) |
| 243116      | 215645314       | A    | -   | frameshift deletion | E429fs         | rs879258379 | -                          | not provided | Pathogenic (Likely pathogenic (Last reviewed: 11 December 2015) |
| 234190      | 215645328       | A    | -   | frameshift deletion | R424fs         | rs876660911 | -                          | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 2 October 2015) |
| 187182      | 215645331-215645334 | frameshift indel | V422fs | rs786203533 | -                          | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 10 November 2014) |
| 229677      | 215645382       | C    | T   | nonsense     | R406 *          | rs377153250 | 0.00003                    | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 11 November 2014) |
| 142734      | 215645386       | C    | G   | nonsense     | Y404 *          | rs587782681 | -                          | Familial cancer of breast | not provided | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 13 October 2016) |
Table 1. Cont.

| Variation ID | GRCh37 Location | Ref. | Alt | Mutation Type | Protein Change | dbSNP | Frequency in ExAC Database | Condition(s) | Clinical Significance (Last Reviewed) |
|--------------|-----------------|------|-----|---------------|----------------|-------|--------------------------|--------------|--------------------------------------|
| 379884       | 215645395       | C    | G   | nonsense      | S402 *         |       | not provided             | Pathogenic    | Last reviewed: 1 June 2015             |
| 187646       | 215645400       | A    |     | frameshift deletion | S400fs        |       | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 17 December 2014        |
| 237814       | 215645426       | C    | G   | nonsense      | S391 *         |       | Familial cancer of breast | Pathogenic    | Last reviewed: 24 February 2016        |
| 185916       | 215645537       | C    | G   | nonsense      | S354 *         |       | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 24 March 2016           |
| 232902       | 215645575       | G    |     | frameshift deletion | S342fs        |       | not provided | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 21 July 2015             |
| 419156       | 215645584       | C    |     | frameshift deletion | S339fs        |       | not provided             | Pathogenic    | Last reviewed: 1 June 2015             |
| 141412       | 215645651       | T    | G   | nonsense      | L316 *         |       | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 7 April 2014             |
| 185015       | 215645737-215645738 | AG |     | frameshift deletion | E287fs        |       | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 29 May 2014              |
| 232418       | 215645759-215645760 | TT |     | frameshift deletion | L280fs        |       | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 15 June 2015             |
| 406737       | 215645833       | A    |     | frameshift duplication | I249fs        |       | Familial cancer of breast | Pathogenic    | Last reviewed: 24 October 2016          |
| 141005       | 215645865       | C    | T   | nonsense      | Q245 *         |       | not provided | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 8 March 2017              |
| 230107       | 215645889       | C    | T   | nonsense      | Q237 *         |       | not provided | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 11 July 2016              |
| 141342       | 215645938-215645941 | TT TA |     | frameshift deletion | R219fs        |       | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 23 February 2014          |
| 219726       | 215645970-215645971 | AA |     | frameshift deletion | K209fs        |       | Familial cancer of breast | Pathogenic/Likely pathogenic | Last reviewed: 4 December 2015            |
| 127742       | 215645975       | A    |     | frameshift deletion | K209fs        |       | Familial cancer of breast | Pathogenic    | Last reviewed: 18 November 2016          |
| 182042       | 215645991       | G    | T   | nonsense      | G203 *         |       | not provided | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 25 September 2014         |
| 232365       | 215646006       | G    |     | frameshift deletion | A198fs        |       | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 15 September 2015         |
| 186576       | 215646058-215646059 | AT |     | frameshift deletion | Y180fs        |       | Familial cancer of breast | Pathogenic    | Last reviewed: 13 December 2016          |
| 406763       | 215646072       | C    | T   | nonsense      | Q176 *         |       | not provided | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 26 May 2016              |
| 185864       | 215646102       | C    | T   | nonsense      | Q166 *         |       | not provided | Hereditary cancer-predisposing syndrome | Pathogenic    | Last reviewed: 4 April 2016              |
Table 1. Cont.

| Variation ID | GRCh37 Location | Ref. | Alt | Mutation Type | Protein Change | Frequency in ExAC Database | Condition(s) | Clinical Significance (Last Reviewed) |
|--------------|-----------------|------|-----|---------------|-----------------|-----------------------------|--------------|---------------------------------------|
| 230344       | 215646138-215646141 | -    | AAAG | frameshift duplication | V154fs | rs772486760 | - | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 2 February 2015) |
| 182036       | 215646150       | C    | T   | nonsense      | R150 * | rs730881411 | 0.00001 | Familial cancer of breast | not provided | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 29 July 2016) |
| 185399       | 215657051       | C    | T   | nonsense      | R112 * | rs758972589 | 0.00001 | Familial cancer of breast | not provided | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 27 May 2016) |
| 185079       | 215657087       | C    | T   | nonsense      | Q100 * | rs786201912 | - | Familial cancer of breast | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 13 December 2016) |
| 230447       | 215657108       | C    | T   | nonsense      | Q93 *  | rs876658971 | - | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 23 February 2015) |
| 231019       | 215657170       | G    | A   | splice acceptor | -       | rs876658005 | - | Hereditary cancer-predisposing syndrome | Likely pathogenic (Last reviewed: 26 March 2015) |
| 371931       | 215661823-215661824 | AG   | -   | frameshift deletion | E99fs | rs1057517589 | - | Familial cancer of breast | Pathogenic/Likely pathogenic (Last reviewed: 16 November 2016) |
| 246176       | 215661842       | G    | T   | splice acceptor | -       | rs879254139 | - | not provided | Likely pathogenic (Last reviewed: 3 December 2015) |
| 246476       | 215674192       | G    | A   | nonsense      | W34 *  | rs879254280 | - | not provided | Likely pathogenic (Last reviewed: 8 March 2016) |
| 231232       | 215674224-215674225 | frameshift indel | A25fs | - | - | rs87659040 | - | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 25 March 2015) |
| 233594       | 215674239       | G    | T   | nonsense      | E19 *  | rs752514155 | 0.00002 | Familial cancer of breast | not provided | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 28 August 2016) |
| 232926       | 215674271-215674290 | frameshift deletion | P26s | - | - | rs876660077 | - | Hereditary cancer-predisposing syndrome | Pathogenic (Last reviewed: 10 July 2015) |
| 233594       | 215674291       | G    | A   | missense      | M1H | rs876700031 | - | not provided | Pathogenic (Last reviewed: 5 November 2013) |
| 154206       | 190300875-242783384 | copy number gain (2q32.2-37.3) | - | - | nsv3395386 | - | Hypospadias | Pathogenic (Last reviewed: 18 March 2014) |
| 152738       | 193803352-216569775 | copy number loss (2p23.3-35) | - | - | nsv1408180 | - | Developmental delay AND/OR other significant developmental or morphological phenotypes | Pathogenic (Last reviewed: 14 January 2013) |
| 150620       | 181378520-225167565 | copy number gain (2q31.3-36.1) | - | - | nsv1604018 | - | Developmental delay AND/OR other significant developmental or morphological phenotypes | Pathogenic (Last reviewed: 25 July 2011) |
| Variation ID | GRCh37 Location | Ref. | Alt | Mutation Type | Protein Change | dbSNP   | Frequency in ExAC Database | Condition(s)                                                   | Clinical Significance (Last Reviewed)                                                                 |
|--------------|-----------------|------|-----|---------------|----------------|---------|-----------------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| 146683       | 211444400–243059659 | .    | .   | copy number gain (2q34-37.3) | -              | nssv756486   | -                           | Coarctation of aorta                                           | Pathogenic (Last reviewed: 25 February 2011)                                                         |
| 59164        | 213479146–227985946 | .    | .   | copy number gain (2q34-36)    | -              | nssv578841   | -                           | Developmental delay AND/OR other significant developmental or morphological phenotypes | Pathogenic (Last reviewed: 12 August 2011)                                                          |
| 59160        | 19387039–242014395 | .    | .   | copy number gain (2q32.3-37.3) | -              | nssv578835   | -                           | Developmental delay AND/OR other significant developmental or morphological phenotypes | Pathogenic (Last reviewed: 12 August 2011)                                                          |
| 59159        | 191173462–242834921 | .    | .   | copy number gain (2q32.2-37.3) | -              | nssv578834   | -                           | Developmental delay AND/OR other significant developmental or morphological phenotypes | Pathogenic (Last reviewed: 12 August 2011)                                                          |
| 59158        | 189662921–243007359 | .    | .   | copy number gain (2q32.2-37.3) | -              | nssv578833   | -                           | Developmental delay AND/OR other significant developmental or morphological phenotypes | Pathogenic (Last reviewed: 12 August 2011)                                                          |
| 57419        | 195763007–237382356 | .    | .   | copy number gain (2q32.3-37.3) | -              | nssv578837   | -                           | Developmental delay AND/OR other significant developmental or morphological phenotypes | Pathogenic (Last reviewed: 12 August 2011)                                                          |

*: truncated protein.
2.2. Common Predisposing Variants of BARD1 in Cancer

Many genome-wide association studies (GWAS), using high-density single nucleotide polymorphism (SNP)-based microarray technology, have been conducted in the commonest cancer types and have identified more than 4032 genetic associations (GWAS catalog, date: 21 August 2017), confirming that susceptibility to these diseases is polygenic. We have performed a large GWAS to define the genetic landscape of sporadic neuroblastoma predisposition and have identified common DNA alleles in different genes [38–47] that are associated significantly with neuroblastoma development. In that GWAS, one of the most significant and robustly replicated association signals that was enriched in the high-risk subset of neuroblastomas resided in the BARD1 locus [21] that is also the only neuroblastoma susceptibility gene validated in Afro-American [48], Chinese [49] and Spanish individuals [50]. We have demonstrated that, in BARD1 locus, SNPs associated with risk of neuroblastoma correlates with high expression of splice variants of BARD1 and SNPs protecting against neuroblastoma correlates with high expression of FL BARD1 [21]. Interestingly, one disease-associated variant (rs6435862) correlates with the expression of an oncogenetically activated isoform, BARD1β, which has growth-promoting effects in neuroblastoma models potentially through cooperation with the Aurora family of kinases [51]. Furthermore, by performing a fine mapping analysis of BARD1 locus, we have identified additionally functional polymorphisms associated with risk of neuroblastoma and over-expression of FL BARD1 [50]. These data strongly suggest that the dual role of BARD1 as oncogene or tumor-suppressor is due to the function of disease-associated variants. Together, these evidences highlight that the risk of neuroblastoma development may be estimated by a specific combination of BARD1 risk genotypes as suggested by the results of a published computational analysis of GWAS-identified neuroblastoma risk loci [52].

Recently, the SNP rs7583536 previously associated to neuroblastoma has been found also associated to nephroblastoma [53], which is the most frequent malignant renal tumor in children. Although the SNP rs7583536 located in 3' UTR of BARD1 may have a role in BARD1 mRNA regulation, additional investigations are needed to validate this genetic association.

Candidate gene association studies have suggested that the low-frequency variant Cys557Ser (rs28997576) confers risk of single and multiple primary breast cancers in Icelandic [26] and South American [54] populations. However, independent studies failed to replicate that genetic association in Polish [55], multiethnic [36], Chinese [57], Australian [58] individuals. We also failed to validate that genetic association in a case series consisting of 540 high-risk neuroblastoma cases and 1142 controls [46] with European-American origins. These discordant results might be due to population substructure or gene modifiers affecting the role of BARD1 in cancer development.

2.3. Somatic Mutations of BARD1 in Cancer

Whereas common and rare hereditable variants of BARD1 have been associated with cancer risk, recent high-throughput sequencing studies have found no frequently acquired somatic mutations in tumor tissues. In accord to previous studies, our exome and deep sequencing of 82 clinically aggressive neuroblastomas detected only one somatic acquired mutation [32]. Interestingly, a large whole exome sequencing study on 500 metastatic cancers identified BARD1 among the genes somatically altered at low-frequency [59] and recently BARD1 has been included in the list of Cancer Gene Census in COSMIC database (http://cancer.sanger.ac.uk/census/). Here we have analyzed all somatic mutations of BARD1 deposited in COSMIC database by using the Cancer-specific High-throughput Annotation of Somatic Mutations (CHASM) [60] tool to distinguish passenger variation events from driver ones across a cohort of tumors and the Variant Effect Scoring Tool (VEST) [61] to identify variants that affect the molecular function of the protein and prioritize them on the basis of the likelihood of their involvement in human disease (Table 2). We confirm that even if pathogenic somatic mutations are relatively infrequent, BARD1 can be considered a cancer driver gene (CHASM gene score = 0.73; CHASM gene p-value = 0.0000004).
| Position     | Sequence Ontology | Protein Sequence | CHASM p-Value | VEST p-Value | ID dbsNP     | Frequency in ExAC Database | COSMIC Variant Count in Tissues (n)                  |
|--------------|-------------------|------------------|---------------|--------------|--------------|----------------------------|------------------------------------------------------|
| 214769310    | SG                | R123 *           | 0.1290        |              | rs369986649  | 0.00                       | large_intestine (1); lung (1); endometrium (1); skin(2) |
| 214781072    | MS                | E268K            | 0.2724        | 0.4362       |              | 0.00                       | cervix(1); liver(2)                                  |
| 214728840    | MS                | A724T            | 0.0462        | 0.0145       |              | 0.00                       | large_intestine(2)                                  |
| 214730417    | MS                | E665D            | 0.3204        | 0.5076       |              | 0.00                       | liver(2)                                             |
| 214728936    | MS                | I692F            | 0.4718        | 0.2635       |              | 0.00                       | liver(2)                                             |
| 214781454    | MS                | K140N            | 0.5620        | 0.1730       | rs758749603  | 0.000008                   | large_intestine(1); endometrium(1)                   |
| 214781285    | SG                | K197 *           | 0.2026        |              |              | 0.00                       | liver(2)                                             |
| 214781251    | FD                | K288RKL *        | 0.0588        |              |              | 0.000017                   | large_intestine(1)                                  |
| 214780799    | SY                | L359L            |              |              |              | 0.00                       | esophagus(2)                                         |
| 214730452    | MS                | P654S            | 0.0970        | 0.6800       |              | 0.00                       | skin(2)                                              |
| 214792396    | MS                | P99A             | 0.2542        | 0.0534       |              | 0.00                       | pancreas(2)                                          |
| 214728907    | MS                | Q701H            | 0.1730        | 0.1709       |              | 0.00                       | esophagus(1)                                         |
| 214781390    | MS                | S162A            | 0.5712        | 0.2689       |              | 0.00                       | esophagus(1)                                         |
| 214780783    | MS                | S364L            | 0.4934        | 0.7981       |              | 0.00                       | esophagus(2)                                         |
| 214780784    | MS                | S364T            | 0.7164        | 0.3940       | rs201292946  | 0.00                       | esophagus(2)                                         |
| 214780955    | MS                | Y307N            | 0.6760        | 0.5994       |              | 0.00                       | esophagus(1)                                         |
| 214769514    | SS                |                 | 0.0374        |              |              | 0.00                       |                                                      |
| 214781510    | SS                |                 | 0.1037        |              |              | 0.00                       |                                                      |
| 214728990    | SG                | G674 *           | 0.0577        |              |              | 0.00                       |                                                      |
| 214745084    | SG                | W629 *           | 0.0191        |              | rs74446711   | 0.000008                   | lung(1)                                              |
| 214780882    | MS                | P331R            | 0.6070        | 0.2104       |              | 0.00                       | autonomic_ganglia(1)                                |
| 214780655    | MS                | V407M            | 0.2596        | 0.8458       |              | 0.00                       | biliary_tract(1)                                     |
| 214781361    | FD                | see footnote     | 0.2006        |              |              | 0.000016                   | biliary_tract(1)                                     |
| 214781136    | SY                | P246P            |              |              | rs587780859  | 0.000096                   | bone(1)                                              |
| 214781165    | MS                | Q237E            | 0.4816        | 0.8826       | rs587780035  | 0.000096                   | bone(1)                                              |
| 214745777    | SY                | L585L            |              |              |              | 0.00                       | breast(1)                                            |
| 214745791    | MS                | Q581K            | 0.3898        | 0.0927       | rs756165637  | 0.000008                   | breast(1)                                            |
| 214792394    | SY                | P89P             |              |              |              | 0.00                       | cervix(1)                                            |
| 214781066    | MS                | E270K            | 0.3180        | 0.3405       |              | 0.00                       | cervix(1)                                            |
| 214781210    | MS                | A222S            | 0.8046        | 0.7358       |              | 0.00                       | endometrium(1)                                       |
| 214781384    | SG                | Q164 *           | 0.6800        | 0.5193       | rs369561166  | 0.000008                   | endometrium(1)                                       |
| 214781306    | MS                | D190Y            | 0.4107        |              |              | 0.00                       | endometrium(1)                                       |
| 214781158    | MS                | L259R            | 0.4934        | 0.5867       |              | 0.00                       | endometrium(1)                                       |
| 214780657    | MS                | R406Q            | 0.6028        | 0.7262       | rs587780014  | 0.0000412                  | endometrium(1)                                       |
| 214730490    | MS                | R641Q            | 0.2660        | 0.6668       | rs752670879  | 0.0000082                  | endometrium(1)                                       |
| 214781449    | SG                | S142 *           | 0.1272        |              |              | 0.00                       | endometrium(1)                                       |
| 214745097    | MS                | L625I            | 0.5980        | 0.2316       |              | 0.00                       | endometrium(1)                                       |
| 214780981    | MS                | V298A            | 0.4174        | 0.9235       |              | 0.00                       | endometrium(1)                                       |
| 214728928    | SY                | L694L            |              |              | rs139620052  | 0.000157                   | endometrium(1)                                       |
| 214745741    | SY                | Y597Y            |              |              |              | 0.00                       | endometrium(1)                                       |
| 214767501    | MS                | G517R            | 0.2520        | 0.0149       |              | 0.00                       | hematopoietic_and_lymphoid_tissue(1)                 |
| Position     | Sequence Ontology | Protein Sequence Change | CHASM p-Value | VEST p-Value | ID dbsNP | ExAC Database | COSMIC Variant Count in Tissues (n) |
|--------------|-------------------|-------------------------|---------------|-------------|----------|---------------|------------------------------------|
| 214767558    | MS                | L498I                   | 0.1770        | 0.0629      |          | 0.00          |                                    |
| 214730463    | MS                | K650T                   | 0.3204        | 0.2411      |          | 0.00          |                                    |
| 214767483    | MS                | V532I                   | 0.2358        | 0.4735      |          | 0.00          |                                    |
| 214767560    | MS                | P497L                   | 0.0730        | 0.0046      |          | 0.00          |                                    |
| 214781275    | MS                | A200V                   | 0.3100        | 0.7394      |          | 0.00          |                                    |
| 214792349    | MS                | M104I                   | 0.1630        | 0.0450      | rs752133770 | 0.000008 | kidney(1)                          |
| 214728757    | SY                | R751R                   | 0.000000000   | 0.000000000 | rs750001065 | 0.000000000 | kidney(1)                          |
| 214728786    | MS                | L742V                   | 0.4234        | 0.7816      |          | 0.00          |                                    |
| 214728839    | MS                | A724V                   | 0.0356        | 0.0153      | rs587782662 | 0.0000041 | large_intestine(1)                 |
| 214728971    | SG                | W680 *                  | 0.0967        | 0.000000000 |          | 0.00          |                                    |
| 214745779    | MS                | L585F                   | 0.2630        | 0.0431      |          | 0.00          |                                    |
| 214752448    | MS                | V559A                   | 0.0894        | 0.2909      |          | 0.00          |                                    |
| 214767651    | MS                | E467K                   | 0.1858        | 0.0168      |          | 0.00          |                                    |
| 214780632    | MS                | M414I                   | 0.7050        | 0.5045      |          | 0.00          |                                    |
| 214780885    | MS                | S330I                   | 0.6160        | 0.6216      |          | 0.00          |                                    |
| 214780904    | MS                | H324N                   | 0.6444        | 0.6244      |          | 0.00          |                                    |
| 214780960    | MS                | K305T                   | 0.7818        | 0.5088      |          | 0.00          |                                    |
| 214780974    | SY                | P300P                   | 0.000000000   | 0.000000000 |          | 0.00          |                                    |
| 214781327    | MS                | V183L                   | 0.5944        | 0.3666      |          | 0.00          |                                    |
| 214781495    | MS                | K127E                   | 0.7706        | 0.7394      |          | 0.00          |                                    |
| 214792327    | SG                | R112 *                  | 0.0323        | 0.000000000 | rs758972589 | 0.0000008 | large_intestine(1)                 |
| 214752523    | MS                | T534K                   | 0.3664        | 0.0564      |          | 0.00          |                                    |
| 214752535    | MS                | P530L                   | 0.0214        | 0.0078      |          | 0.00          |                                    |
| 214781357    | SY                | L239L                   | 0.000000000   | 0.000000000 | rs760951875 | 0.0000009 | liver(1)                           |
| 214781422    | MS                | S151N                   | 0.3204        | 0.1398      |          | 0.00          |                                    |
| 214792355    | MS                | D102E                   | 0.5138        | 0.1463      |          | 0.00          |                                    |
| 2147809564   | SY                | P2P                     | 0.000000000   | 0.000000000 |          | 0.00          |                                    |
| 214730432    | MS                | S660R                   | 0.2322        | 0.0874      |          | 0.00          |                                    |
| 214754727    | MS                | S602I                   | 0.6660        | 0.1905      |          | 0.00          |                                    |
| 214743805    | MS                | G576V                   | 0.4174        | 0.1383      |          | 0.00          |                                    |
| 214752450    | SY                | S558S                   | 0.5474        | 0.5937      |          | 0.00          |                                    |
| 214767507    | MS                | S515A                   | 0.6334        | 0.5355      |          | 0.00          |                                    |
| 214767382    | MS                | T490P                   | 0.4354        | 0.5565      |          | 0.00          |                                    |
| 214780037    | MS                | A413T                   | 0.3100        | 0.0492      |          | 0.00          |                                    |
| 214780042    | MS                | P411H                   | 0.3420        | 0.0515      |          | 0.00          |                                    |
| 214780818    | SY                | V352V                   | 0.5045        | 0.000000000 | rs768469265 | 0.0000008 | lung(1)                            |
| 214780842    | MS                | S344R                   | 0.6530        | 0.5045      |          | 0.00          |                                    |
| 214780856    | MS                | I340F                   | 0.8810        | 0.8826      |          | 0.00          |                                    |
| 214781201    | MS                | E225K                   | 0.2884        | 0.6445      |          | 0.00          |                                    |
| 214781353    | MS                | S174I                   | 0.7774        | 0.7572      |          | 0.00          |                                    |
Table 2. Cont.

| Position    | Sequence Ontology | Protein Sequence Change | CHASM p-Value | VEST p-Value | ID dbSNP       | ExAC Database | COSMIC Variant Count in Tissues (n) |
|-------------|------------------|-------------------------|---------------|-------------|----------------|---------------|------------------------------------|
| 214781387   | MS               | V163M                   | 0.2284        | 0.3354      | 0.00           | lung(1)       |                                    |
| 214790704   | MS               | H68Y                    | 0.0190        | 0.0139      | 0.00           | lung(1)       |                                    |
| 214699069   | MS               | R21C                    | 0.2122        | 0.7193      | 0.00           | lung(1)       |                                    |
| 214769252   | MS               | H459Y                   | 0.2160        | 0.2473      | 0.00           | NS(1)         |                                    |
| 214769253   | SY               | D458D                   | 0.00          |             | 0.00           | NS(1)         |                                    |
| 214769270   | MS               | D453N                   | 0.2630        | 0.1696      | 0.00           | NS(1)         |                                    |
| 214809491   | MS               | E27K                    | 0.1308        | 0.5288      | 0.00           | NS(1)         |                                    |
| 214728731   | MS               | S760L                   | 0.2122        | 0.0823      | rs730881425    | 0.000025      | esophagus(1)                      |
| 214728819   | MS               | R731C                   | 0.2852        | 0.3666      | rs76744638     | 0.000041      | esophagus(1)                      |
| 214745127   | MS               | Q615E                   | 0.4234        | 0.1915      | rs751710099    | 0.000008      | esophagus(1)                      |
| 214767850   | SY               | S515S                   | 0.00          |             |                | esophagus(1)  |                                    |
| 214767573   | MS               | Q493E                   | 0.5370        | 0.5719      | 0.00           | esophagus(1)  |                                    |
| 214780709   | MS               | S389R                   | 0.5944        | 0.4714      | 0.00           | esophagus(1)  |                                    |
| 214781120   | MS               | F252S                   | 0.3204        | 0.8220      | rs758368819    | 0.000009      | esophagus(1)                      |
| 21478351    | MS               | A173S                   | 0.8810        | 0.4547      | 0.00           | esophagus(1)  |                                    |
| 214745744   | SY               | K966K                   | 0.1042        | 0.0035      | 0.00           | pancreas(1)   |                                    |
| 214752510   | SY               | S538S                   | 0.4354        | 0.2288      | 0.00           | pancreas(1)   |                                    |
| 214752635   | MS               | G472E                   | 0.0304        | 0.0144      | 0.00           | prostate(1)   |                                    |
| 214792385   | MS               | I92M                    | 0.6340        | 0.7262      | rs774251286    | 0.000017      | pituitary(1)                     |
| 214769009   | MS               | R322H                   | 0.1114        | 0.5492      | 0.00           | prostate(1)   |                                    |
| 214738471   | SY               | E665E                   | 0.0000        |             | 0.00           | prostate(1)   |                                    |
| 214752536   | MS               | P530S                   | 0.0144        | 0.0102      | rs76014424    | 0.000008      | prostate(1)                      |
| 214769261   | MS               | V456I                   | 0.1904        | 0.4630      | 0.00           | prostate(1)   |                                    |
| 214781033   | MS               | P281S                   | 0.1338        | 0.6871      | rs200059956    | 0.000017      | prostate(1)                      |
| 214781127   | SY               | I249I                   | 0.00          |             | rs764551077    | 0.000009      | prostate(1)                      |
| 214783355   | SY               | A173A                   | 0.00          |             | 0.00           | prostate(1)   |                                    |
| 214772872   | MS               | R566R                   | 0.1730        | 0.2288      | 0.00           | skin(1)       |                                    |
| 214730442   | MS               | P567L                   | 0.0304        | 0.0144      | 0.00           | skin(1)       |                                    |
| 214730443   | MS               | P657S                   | 0.1270        | 0.1673      | 0.00           | skin(1)       |                                    |
| 214745129   | MS               | V614A                   | 0.1114        | 0.5492      | 0.00           | skin(1)       |                                    |
| 214743834   | SY               | R566R                   | 0.00          |             | 0.00           | skin(1)       |                                    |
| 214767638   | MS               | T489I                   | 0.1730        | 0.2288      | 0.00           | skin(1)       |                                    |
| 214767639   | MS               | H471Y                   | 0.2284        | 0.1872      | rs867587389    | 0.00          | skin(1)                           |
| 214769288   | MS               | L447F                   | 0.1204        | 0.0426      | 0.00           | skin(1)       |                                    |
| 214780870   | MS               | A435V                   | 0.3420        | 0.0168      | 0.00           | skin(1)       |                                    |
| 214780869   | MS               | V422G                   | 0.8136        | 0.8197      | rs76824305    | 0.000232      | skin(1)                           |
| 214780843   | MS               | P411S                   | 0.0962        | 0.1509      | 0.00           | skin(1)       |                                    |
| 214780882   | MS               | P331L                   | 0.3898        | 0.2518      | 0.00           | skin(1)       |                                    |
| 214781320   | MS               | P18SL                   | 0.4934        | 0.8492      | 0.00           | skin(1)       |                                    |
| 214781323   | MS               | S184F                   | 0.1858        | 0.2233      | rs184660818    | 0.00          | skin(1)                           |
| 214792395   | MS               | P98Q                    | 0.1056        | 0.0249      | 0.00           | skin(1)       |                                    |
| 214792396   | MS               | P98S                    | 0.0212        | 0.0417      | 0.00           | skin(1)       |                                    |
Table 2. Cont.

| Position   | Sequence Ontology | Protein Sequence Change | CHASM p-Value | VEST p-Value | ID dbSNP          | Frequency in ExAC Database | COSMIC Variant Count in Tissues (n)       |
|------------|-------------------|-------------------------|---------------|-------------|-------------------|-----------------------------|------------------------------------------|
| 214809508  | MS                | R21L                    | 0.2038        | 0.6894      |                   | 0.00                        | skin(1)                                 |
| 214728752  | MS                | G733D                   | 0.0762        | 0.0168      | rs867281641       | 0.00                        | stomach(1)                             |
| 214728780  | MS                | N744D                   | 0.5416        | 0.4141      |                   | 0.00                        | stomach(1)                             |
| 214728831  | MS                | D727N                   | 0.3044        | 0.5867      | rs730881424       | 0.000025                    | stomach(1)                             |
| 214728891  | MS                | P707S                   | 0.0436        | 0.0289      |                   | 0.00                        | stomach(1)                             |
| 214728985  | SY                | C675C                   |               |             |                   | 0.00                        | stomach(1)                             |
| 214730411  | SY                | L667L                   |               |             |                   | 0.00                        | stomach(1)                             |
| 214767511  | SY                | L513L                   |               |             |                   | 0.00                        | stomach(1)                             |
| 214767611  | MS                | L480S                   | 0.1242        | 0.0086      | rs149839922       | 0.000008                    | stomach(1)                             |
| 214769254  | MS                | D458V                   | 0.0432        | 0.0034      |                   | 0.00                        | stomach(1)                             |
| 214780751  | MS                | T375A                   | 0.5088        | 0.8954      |                   | 0.00                        | stomach(1)                             |
| 214781250  | FI                | K208KENFS *             | 0.0618        |             | rs587780033       | 0.000017                    | stomach(1)                             |
| 214781418  | SY                | K152K                   |               |             |                   | 0.00                        | stomach(1)                             |
| 214792416  | MS                | G82V                    | 0.4234        | 0.2775      |                   | 0.00                        | stomach(1)                             |
| 214745083  | MS                | W629C                   | 0.1678        | 0.0025      |                   | 0.00                        | urinary_tract(1)                       |
| 214781258  | MS                | Q206K                   | 0.4174        | 0.6353      |                   | 0.00                        | urinary_tract(1)                       |
| 214728689  | MS                | P774L                   | 0.3692        | 0.1073      |                   | 0.00                        | urinary_tract(1)                       |
| 214745730  | MS                | D601G                   | 0.6122        | 0.1612      | rs767765131       | 0.000006                    | upper_aerodigestive_tract(1)            |
| 214769271  | MS                | S452R                   | 0.5370        | 0.1476      |                   | 0.00                        | upper_aerodigestive_tract(1)            |
| 214781182  | MS                | S231C                   | 0.5654        | 0.5912      |                   | 0.00                        | upper_aerodigestive_tract(1)            |
| 214809454  | MS                | A39G                    | 0.5572        | 0.4215      |                   | 0.00                        | urinary_tract(1)                       |
| 214745154  | MS                | H606D                   | 0.1552        | 0.0017      |                   | 0.00                        | urinary_tract(1)                       |
| 214781269  | MS                | S202C                   | 0.3982        | 0.4655      |                   | 0.00                        | urinary_tract(1)                       |

SG: Stop Gain; MS: Missense; FD: Frameshift Deletion; SY: Synonymous; SS: Splicing Site; FI: Frameshift Insertion; *: truncated proteins; ~: protein deletion
K171KMQVLSKTMNLFPQVLLQMFRLGRKLQDLEKSKKRL; ID dbSNP: Nomenclature of the single nucleotide polymorphism; Exac database (http://exac.broadinstitute.org/): Exome Aggregation Consortium is a database that reports the frequency of variants from a wide variety of large-scale sequencing projects, CHASM: Cancer-specific High-throughput Annotation of Somatic Mutations; VEST: Variant Effect Scoring Tool.
3. Biological Functions of BARD1 as Tumor Suppressor

Tumor suppressor functions of BRCA1 are thought to be mediated by the BARD1-BRCA1 heterodimer which is an E3 ubiquitin ligase implicated in DNA repair [18,19] and in other essential functions for maintaining genomic stability [62,63], as homologous recombination [64], centrosome duplication [62] and mitotic spindle assembly [65] (Figure 3). Specific functions of BARD1-BRCA1 heterodimer will not be dealt with in this paragraph. Although partner of this complex, FL BARD1 initiates or facilitates DNA repair pathways by controlling polyadenylation machinery in BRCA1-independent way through BARD1 binding with mRNA polyadenylation factor cleavage stimulation factor (CSTF1) [66,67].

Figure 3. Full-length (FL) BARD1 pathways and functions. FL BARD1 participates in two major pathways as tumor suppressor. (A) BRCA1-independent pathways are mediated by the interaction of BARD1 with proteins involved in oncogenic pathways. BARD1 has transcriptional activity as it can induce the transcription activity of NF-κB through binding to the NF-κB co-factor BCL3 [68]. FL BARD1 interacts to poly(ADP-ribose) (PAR) after damage and consequently it is recruited to DNA repair [69]. Finally, increased expression levels of FL BARD1 stabilize p53 and facilitate its phosphorylation by DNA-dependent protein kinase (DNAPK) [70–72]; (B) BRCA1-dependent pathways are mediated by BARD1-BRCA1 heterodimer. The activity of the BARD1-BRCA1 ubiquitin ligase is implicated in essential functions for maintaining genomic stability [61–64].

BARD1 expression fluctuates in a cell-cycle dependent manner, with maximal expression levels occurring in mitosis [73]. In mitosis FL BARD1 stability is increased due to phosphorylation by cell-cycle dependent kinase complexes (cyclin A/E-CDK2 and cyclin B-CDK2) within regions required for ubiquitin ligase activity of BARD1-BRCA1 heterodimer [74]. Contrary, BRCA1 is mostly expressed during S-phase of cell-cycle [73]. We can speculate that the concomitant expression of BARD1 and BRCA1 in S-phase support the function of BARD1-BRCA1 heterodimer and FL BARD1 expression in mitosis supports additional BRCA1-independent functions.
BRCA1 and BARD1 have specific individual functions due to their interaction with various proteins and the dissociation of heterodimer might be regulated by post-translation protein modifications such as phosphorylation, ubiquitination or PARylation. Cancer-associated BRCA1-independent activities of BARD1 have been reported in various tumor cell lines (Figure 3). An access of monomer BARD1 over BRCA1 has been associated with BRCA1 mutations and with p53-mediated apoptosis. The link between BARD1 and apoptosis has been further highlighted by BARD1 co-immunoprecipitation with p53 in tissues exposed to genotoxic stress [70,71]. Particularly, the region of BARD1 binding with p53 involves ANK repeats and the region between ANK and BRCT domains in BARD1-C terminal fragment [72]. It is interesting to note that mutations or deletions in TP53 gene are frequent in cancer with BRCA1 mutations [75,76]. We can speculate that BRCA1 mutated tumors save BARD1 pro-apoptotic functions and additional TP53 mutations may enhance cancer development. Contrary deleterious BARD1 mutations are infrequent in cancer because the cells lose both DNA repair capabilities and pro-apoptotic function. BARD1 is also transcriptionally up-regulated in response to genotoxic stress and in brain after hypoxia suggesting that BARD1 is expressed specifically in tissues undergoing apoptosis [71].

BARD1 is involved in transcription factor NF-κB pathway. The binding of C-terminal fragment of BARD1 to the ANK repeats domain of BCL3, a NF-κB inhibitor in vitro, may affect the correct regulation of NF-κB in cancer and inflammatory and autoimmune diseases [68]. Emerging evidences report the interaction of BARD1 BRCT domain to poly(ADP-ribose) (PAR) and consequent recruitment of BARD1-BRCA1 complex to DNA repair after damage [69]. PAR pathway is particularly interesting because of the promising drugs act on inhibiting PAR polymerizing enzyme (PARP) are more efficient in cells BRCA1 mutated with saved BARD1 tumor suppressor function. Finally, a significant association found between over-expression of FL BARD1 and favorable outcome in colon cancer patients highlighted FL BARD1 function as prognostic factor in cancer [20].

4. Biological Functions of BARD1 as Oncogene

BARD1 is characterized by full length and diverse spliced isoforms (Figure 1). Down-regulation of FL BARD1 can have oncogenic effects [5,11,20,21] whereas BARD1 isoforms that lack RING or/and ANK domains are often up-regulated and associated with negative prognosis in breast [15], ovarian [15] endometrial [77] and lung [78] cancers. Several scientific evidences show that cancer-associated BARD1 isoforms antagonize the functions of FL BARD1 as tumor suppressor and act as a driving force for carcinogenesis.

BARD1β and BARD1δ isoforms were first identified in rat spermatocytes and in a highly tumorigenic and resistant to apoptosis rat ovarian cancer cell line NuTu-19 [70,79]. BARD1β is characterized by lack of exons 2 and 3 and encode to a protein lacking the RING finger and BRCA1 domain interaction. In breast and ovarian cancer an imbalance of FL BARD1 and BARD1β was observed with BARD1β dominant negative function. BARD1β scaffolds Aurora B and BRCA2 at the midbody during telophase and cytokinesis, antagonizing Aurora B ubiquitination and degradation by BARD1-BRCA1 E3 ubiquitin ligase [22]. BARD1β oncogenic driver of tumorigenesis is also supported by GWAS that identified BARD1 as new susceptibility locus in neuroblastoma as mentioned above [51]. BARD1β depletion in vitro caused genotype-specific inhibition of cell proliferation in neuroblastoma cells, whereas overexpression of BARD1β led to the transformation of non-malignant murine fibroblast [51,77].

BARD1δ is characterized by deletion of exons 2–6 that encode for the majority of the RING finger and the entirety of the ANK repeats, critical regions for the interaction with BRCA1 and p53; this isoform was detected in many gynecological cancers and in multiple processes of tumorigenesis [70,80,81]. In MCF-7 cells, BARD1δ does not stimulate apoptosis due to p53 deficiency [80]; however, its mitochondrial localization suggested a function in regulation of mitochondrial response to tumorigenic stress [82]. Interestingly, BARD1δ specifically binds to estrogen receptor alpha (ERα) antagonizing ERα-BARD1 binding and ERα degradation [83]. To note, BARD1δ
dominant negative of FL BARD1 is temporally and spatially regulated by estrogen signaling in human invasive cytotrophoblasts cells of early pregnancy [81]. Recently, Maxim Pilyugin et al. described BARD1δ antagonizes chromosome and telomere protection function of BARD1-BRCA1 heterodimer by binding molecules that confer chromosome integrity [84]. It is likely that BARD1δ confers genomic instability and acquired oncogenic property in absence of cell cycle control, due to p53 deficiency and of chromosome integrity.

BARD1ω isoform contains only exons 6–11 encoding ANK repeats and BRCT domain. This isoform was found highly expressed in acute myeloid leukemia (AML) and in AML cell lines. In vitro BARD1ω overexpression induced multiple mitotic defects like aberrant chromosome alignment at the metaphase and anaphase state, abnormally increased size of nucleus and apoptosis inhibition. These scientific evidences highlight oncogenic proprieties of BARD1ω [85].

5. Summary and Future Perspectives

In this review, we summarized the genetic and molecular mechanisms associated to a dualistic role of BARD1 in cancer initiation: tumor suppressor and oncogene. BARD1 shows relatively low frequent mutations in cancer and, even if rare, BARD1 mutations seem to drive malignant transformation. The reduced expression of FL BARD1 due to somatic mutations or predisposition gene silencing variants may be considered the first hit of BARD1 tumor suppressor function. Instead, FL BARD1 loss-of-function consequently to aberrant splicing and gain of dominant negative functions is associated with its proto-oncogenic role. Indeed, cancer associated BARD1 isoforms antagonize the functions of FL BARD1 as tumor suppressor and lead to genetic instability, loss of DNA repair and cell cycle control functions and permits uncontrolled proliferation. This antagonist effect is also supported from a more recently published research article that suggests that specific microRNAs, in healthy tissues, maintain an equilibrium of FL BARD1 and isoforms in favor of FL BARD1 instead, in cancer cells, create a disequilibrium in favor of BARD1 isoforms upon epigenetic activation of non-coding BARD1 isoform BARD1 9’L [23].

In ClinVar database, beyond deletion, nonsense or frame shift mutations, only one missense mutation of BARD1 is reported as “Pathogenic” even if recent literature demonstrates the association of common and rare point mutations with cancer initiation [29,86]. Thus, further functional investigations of non-coding and coding disease-associated variants are needed in order to verify their role in tumorigenesis and drug response.

BARD1 might also play a role in early organogenesis and in diseases related to tissues that originate from neural crest cells. In light of these evidences additional studies to explore BARD1 function in cancer and in developmental disorders should be considered in the next future.

BARD1 as Possible Biomarker and Therapeutic Possibilities

BARD1β has been identified as an oncogenic driver of high-risk neuroblastoma tumorigenesis and a stabilizer of Aurora family of kinases. This strongly supports the development of potential therapeutic strategy with Aurora kinase inhibitors for clinically aggressive neuroblastoma. Moreover, the switching from FL BARD1 to BARD1β permits the deregulated turnover of the Aurora kinases. Thus, Aurora and BARD1β expression levels might be predictive biomarkers for response to Aurora inhibitors.

Protein PARYlation functions as a signal to recruit DNA damage repair proteins like the BARD1-BRCA1 complex to repair Double Strand Breaks (DSBs). BARD1 BRCTs bind ADP-ribose, the basic unit of PARY, at DNA damage sites which mediates the rapid recruitment of BRCA1. PARY inhibition directly suppresses the fast recruitment of the BARD1-BRCA1 heterodimer to DNA damage sites and impairs DNA repair. PARY inhibitors (PARYI) selectively kill BRCA1-deficient cells and several PARYI are currently in breast cancer clinical trials. However, the mechanism underlying the sensitivity of the tumor cells bearing BRCA1 mutations that abolish the interaction between BRCA1 and BARD1 to PARYI is not clear [87]. Ovarian and breast cancer patients who harbor BRCA1 mutations develop resistance to both PARYI and platinum therapy [88,89]. Secondary mutations in BRCA genes
as well as gene methylation status for BRCA1, BRCA2 and other genes that control homologous recombination have been examined in patients’ biopsies as potential resistance mechanisms. One way to overcome clinical resistance is to investigate as the expression of FL or isoform BARD1 could contribute to the success or failure of PARPi therapy. A recent paper has demonstrated that BARD1β sensitizes colon cancer cells to poly PARP-1 inhibition even in a FL BARD1 background, thus suggesting that BARD1β may serve as a future biomarker to assess suitability of colon cancers for homologous recombination targeting with PARPi in treatment of advanced colon cancer [90]. In the future, it will be interesting to evaluate the efficacy of PARPi in patients with loss-of-function mutations of BARD1 that are relatively frequent (Table 1).

The early detection of cancer is the most important factor contributing to the total eradication of cancer. The over-expression of BARD1 isoforms is strongly correlated with tumor progression, specifically in non-small-cell lung cancer (NSCLC) [20,51,78]. Based on these evidences Irminger-Finger et al. have developed a blood test for the early detection and diagnosis of lung cancer based on capturing autoimmune antibodies against BARD1 antigens [91]. Additional studies are needed to verify the efficacy of this test in detection of lung cancer and it will be very interesting to extend this experimentation to other cancers such as neuroblastoma, ovarian and breast cancer.

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References
1. Wu, L.C.; Wang, Z.W.; Tsan, J.T.; Spillman, M.A.; Phung, A.; Xu, X.L.; Yang, M.C.; Hwang, L.Y.; Bowcock, A.M.; Baer, R. Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nat. Genet. 1996, 14, 430–440. [CrossRef] [PubMed]
2. Brzovic, P.S.; Meza, J.E.; King, M.C.; Klevit, R.E. BRCA1 RING domain cancer-predisposing mutations. Structural consequences and effects on protein-protein interactions. J. Biol. Chem. 2001, 276, 41399–41406. [CrossRef] [PubMed]
3. Irminger-Finger, I.; Ratajska, M.; Pilyugin, M. New concepts on BARD1: Regulator of BRCA pathways and beyond. Int. J. Biochem. Cell Biol. 2016, 72, 1–17. [CrossRef] [PubMed]
4. Irminger-Finger, I.; Jefford, C.E. Is there more to BARD1 than BRCA1? Nat. Rev. Cancer 2006, 6, 382–391. [CrossRef] [PubMed]
5. Irminger-Finger, I.; Soriano, J.V.; Vaudan, G.; Montesano, R.; Sappino, A.P. In Vitro repression of Brca1-associated RING domain gene, Bard1, induces phenotypic changes in mammary epithelial cells. J. Cell Biol. 1998, 143, 1329–1339. [CrossRef] [PubMed]
6. Ayi, T.C.; Tsan, J.T.; Hwang, L.Y.; Bowcock, A.M.; Baer, R. Conservation of function and primary structure in the BRCA1-associated RING domain (BARD1) protein. Oncogene 1998, 17, 2143–2148. [CrossRef] [PubMed]
7. Joukov, V.; Chen, J.; Fox, E.A.; Green, J.B.; Livingston, D.M. Functional communication between endogenous BRCA1 and its partner, BARD1, during Xenopus laevis development. Proc. Natl. Acad. Sci. USA 2001, 98, 12078–12083. [CrossRef] [PubMed]
8. Boulton, S.J.; Martin, J.S.; Polanowska, J.; Hill, D.E.; Gartner, A.; Vidal, M. BRCA1/BARD1 orthologs required for DNA repair in Caenorhabditis elegans. Curr. Biol. 2004, 14, 33–39. [CrossRef] [PubMed]
9. Lafarge, S.; Montane, M.H. Characterization of Arabidopsis thaliana ortholog of the human breast cancer susceptibility gene 1: AtBRCA1, strongly induced by gamma rays. Nucleic Acids Res. 2003, 31, 1148–1155. [CrossRef] [PubMed]
10. Shakya, R.; Szabolcs, M.; McCarthy, E.; Osipina, E.; Basso, K.; Nandula, S.; Murty, V.; Baer, R.; Ludwig, T. The basal-like mammary carcinomas induced by Brca1 or Bard1 inactivation implicate the BRCA1/BARD1 heterodimer in tumor suppression. Proc. Natl. Acad. Sci. USA 2008, 105, 7040–7045. [CrossRef] [PubMed]
11. McCarthy, E.E.; Celebi, J.T.; Baer, R.; Ludwig, T. Loss of Bard1, the heterodimeric partner of the Brca1 tumor suppressor, results in early embryonic lethality and chromosomal instability. *Mol. Cell. Biol.* 2003, 23, 5056–5063. [CrossRef] [PubMed]

12. Ghimenti, C.; Sensi, E.; Prescittini, S.; Brunetti, I.M.; Conte, P.; Bevilacqua, G.; Caligo, M.A. Germline mutations of the BRCA1-associated RING domain (BARD1) gene in breast and breast/ovarian families negative for BRCA1 and BRCA2 alterations. *Genes Chromosomes Cancer* 2002, 33, 235–242. [CrossRef] [PubMed]

13. Ishitobi, M.; Miyoshi, Y.; Hasegawa, S.; Egawa, C.; Tamaki, Y.; Monden, M.; Noguchi, S. Mutational analysis of *BARD1* in familial breast cancer patients in Japan. *Cancer Lett.* 2003, 200, 1–7. [CrossRef]

14. Irminger-Finger, I. BARD1, a possible biomarker for breast and ovarian cancer. *Gynecol. Oncol.* 2010, 117, 211–215. [CrossRef] [PubMed]

15. Deng, C.X. BRCA1: Cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. *Nucleic Acids Res.* 2006, 34, 1416–1426. [CrossRef] [PubMed]

16. Hashizume, R.; Fukuda, M.; Maeda, I.; Nishikawa, H.; Oyake, D.; Yabuki, Y.; Ogata, H.; Ohta, T. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *J. Biol. Chem.* 2001, 276, 14537–14540. [CrossRef] [PubMed]

17. Capasso, M.; Diskin, S.J.; Totaro, F.; Longo, L.; De Mariano, M.; Russo, R.; Cimmino, F.; Hakonarson, H.; Tonini, G.P.; Devoto, M.; et al. Replication of GWAS-identified neuroblastoma risk loci strengthens the role of BARD1 and affirms the cumulative effect of genetic variations on disease susceptibility. *Carcinogenesis* 2013, 34, 605–611. [CrossRef] [PubMed]

18. Norquist, B.M.; Harrell, M.I.; Brady, M.F.; Walsh, T.; Lee, M.K.; Gulsuner, S.; Bernards, S.S.; Casadei, S.; Yi, Q.; Burger, R.A.; et al. Inherited Mutations in Women With Ovarian Carcinoma. *JAMA Oncol.* 2016, 2, 482–490. [CrossRef] [PubMed]

19. Pilyugin, M.; Irminger-Finger, I. Long non-coding RNA and microRNAs might act in regulating the expression of BARD1 mRNAs. *Int. J. Biochem. Cell Biol.* 2014, 54, 356–367. [CrossRef] [PubMed]

20. Norquist, B.M.; Harrell, M.I.; Brady, M.F.; Walsh, T.; Lee, M.K.; Gulsuner, S.; Bernards, S.S.; Casadei, S.; Yi, Q.; Burger, R.A.; et al. Inherited Mutations in Women With Ovarian Carcinoma. *JAMA Oncol.* 2016, 2, 482–490. [CrossRef] [PubMed]

21. Stacey, S.N.; Sulem, P.; Johannsson, O.T.; Helgason, A.; Guzmundsson, J.; Kostic, J.P.; Kristjansson, K.; Jonsdottir, T.; Sigurdsson, H.; Hrafnkelsson, J.; et al. The BARD1 Cys557Ser variant and breast cancer risk in Iceland. *PLoS Med.* 2006, 3, e217. [CrossRef] [PubMed]

22. Guenard, F.; Labrie, Y.; Ouellette, G.; Beauparlant, C.J.; Durocher, F.; BRCA1, I. Genetic sequence variations of BRCA1-interacting genes AURKA, BAP1, BARD1 and DHX9 in French Canadian families with high risk of breast cancer. *J. Hum. Genet.* 2009, 54, 152–161. [CrossRef] [PubMed]

23. De Brakkee, S.; De Greve, J.; Loris, R.; Janin, N.; Lissens, W.; Sermijn, E.; Teugels, E. Cancer predisposing missense and protein truncating BARD1 mutations in non-BRCA1 or BRCA2 breast cancer families. *Hum. Mutat.* 2010, 31, E1175–E1185. [CrossRef] [PubMed]
29. Couch, F.J.; Shimelis, H.; Hu, C.; Hart, S.N.; Polley, E.C.; Na, J.; Hallberg, E.; Moore, R.; Thomas, A.; Liblyquist, J.; et al. Associations between cancer predisposition testing panel genes and breast cancer. *JAMA Oncol.* 2017, 3, 1190–1196. [CrossRef] [PubMed]

30. Ratajska, M.; Antoszewska, E.; Piskorz, A.; Brozek, I.; Borg, A.; Kusmierek, H.; Biernat, W.; Limon, J. Cancer predisposing BARD1 mutations in breast-ovarian cancer families. *Breast Cancer Res. Treat.* 2012, 131, 89–97. [PubMed]

31. Ratajska, M.; Matusiak, M.; Kuzniacka, A.; Wasag, B.; Brozek, I.; Biernat, W.; Koczkowska, M.; Debniak, J.; Sniadecki, M.; Kozlowski, P.; et al. Cancer predisposing BARD1 mutations affect exon skipping and are associated with overexpression of specific BARD1 isoforms. *Oncol. Rep.* 2015, 34, 2609–2617. [CrossRef] [PubMed]

32. Lasorsa, V.A.; Formicola, D.; Pignataro, P.; Cimmino, F.; Calabrese, F.M.; Mora, J.; Esposito, M.R.; Pantile, M.; Zanon, C.; De Mariano, M.; et al. Exome and deep sequencing of clinically aggressive neuroblastoma reveal somatic mutations that affect key pathways involved in cancer progression. *Oncotarget* 2016, 7, 21840–21852. [CrossRef] [PubMed]

33. Pugh, T.J.; Morozova, O.; Attiyeh, E.F.; Asgharzadeh, S.; Wei, J.S.; Auclair, D.; Carter, S.L.; Cibulskis, K.; Hanna, M.; Kiezun, A.; et al. The genetic landscape of high-risk neuroblastoma. *Nat. Genet.* 2013, 45, 279–284. [CrossRef] [PubMed]

34. Silversides, C.K.; Lionel, A.C.; Costain, G.; Merico, D.; Migita, O.; Liu, B.; Yuen, T.; Rickaby, J.; Thiruvahindrapuram, B.; Marshall, C.R.; et al. Rare copy number variations in adults with tetralogy of Fallot implicate novel risk gene pathways. *PLoS Genet.* 2012, 8, e1002843. [CrossRef] [PubMed]

35. Faingold, R.; Babyn, P.S.; Yoo, S.J.; Dipchand, A.I.; Weitzman, S. Neuroblastoma with atypical metastases to cardiac and skeletal muscles: MRI features. *Pediatr. Radiol.* 2003, 33, 584–586. [CrossRef] [PubMed]

36. George, R.E.; Lipshultz, S.E.; Lipsitz, S.R.; Colan, S.D.; Diller, L. Association between congenital cardiovascular malformations and neuroblastoma. *J. Pediatr.* 2004, 144, 444–448. [CrossRef] [PubMed]

37. Caminsky, N.G.; Mucaki, E.J.; Petri, A.M.; Lu, R.; Knoll, J.H.; Rogan, P.K. Prioritizing variants in complete hereditary breast and ovarian cancer genes in patients lacking known BRCA mutations. *Hum. Mutat.* 2016, 37, 640–652. [CrossRef] [PubMed]

38. McDaniel, L.D.; Conkrite, K.L.; Chang, X.; Capasso, M.; Vaksman, Z.; Oldridge, D.A.; Zachariou, A.; Horn, M.; Diamond, M.; Hou, C.; et al. Common variants upstream of MLF1 at 3q25 and within CPZ at 4p16 associated with neuroblastoma. *PLoS Genet.* 2017, 13, e1006787. [CrossRef] [PubMed]

39. Capasso, M.; McDaniel, L.D.; Cimmino, F.; Cirino, A.; Formicola, D.; Russell, M.R.; Raman, P.; Cole, K.A.; Diskin, S.J. The functional variant rs34330 of CDKN1B is associated with risk of neuroblastoma. *J. Cell. Mol. Med.* 2017, 21, 3224–3230. [CrossRef] [PubMed]

40. Oldridge, D.A.; Wood, A.C.; Weichert-Leahy, N.; Crimmins, I.; Sussman, R.; Winter, C.; McDaniel, L.D.; Diamond, M.; Hart, L.S.; Zhu, S.; et al. Genetic predisposition to neuroblastoma mediated by a LMO1 super-enhancer polymorphism. *Nature* 2015, 528, 418–421. [CrossRef] [PubMed]

41. Capasso, M.; Diskin, S.; Cimmino, F.; Aciero, G.; Totaro, F.; Petrosino, G.; Pezone, L.; Diamond, M.; McDaniel, L.; Hakonarson, H.; et al. Common genetic variants in NEFL influence gene expression and neuroblastoma risk. *Cancer Res.* 2014, 74, 6913–6924. [CrossRef] [PubMed]

42. Diskin, S.J.; Capasso, M.; Diamond, M.; Oldridge, D.A.; Conkrite, K.; Bosse, K.R.; Russell, M.R.; Iolascon, A.; Hakonarson, H.; Devoto, M.; et al. Rare variants in *TP53* and susceptibility to neuroblastoma. *J. Natl. Cancer Inst.* 2014, 106. [CrossRef] [PubMed]

43. Diskin, S.J.; Capasso, M.; Schneppe, R.W.; Cole, K.A.; Attiyeh, E.F.; Hou, C.; Diamond, M.; Carpenter, E.L.; Winter, C.; Lee, H.; et al. Common variation at 6q16 within HACE1 and LIN28B influences susceptibility to neuroblastoma. *Nat. Genet.* 2012, 44, 1126–1130. [CrossRef] [PubMed]

44. Nguyen, B.; Diskin, S.J.; Capasso, M.; Wang, K.; Diamond, M.A.; Glessner, J.; Kim, C.; Attiyeh, E.F.; Mosse, Y.P.; Cole, K.; et al. Phenotype restricted genome-wide association study using a gene-centric approach identifies three low-risk neuroblastoma susceptibility Loci. *PLoS Genet.* 2011, 7, e1002026. [CrossRef] [PubMed]

45. Wang, K.; Diskin, S.J.; Zhang, H.; Attiyeh, E.F.; Winter, C.; Hou, C.; Schneppe, R.W.; Diamond, M.; Bosse, K.; Mayes, P.A.; et al. Integrative genomics identifies LMO1 as a neuroblastoma oncogene. *Nature* 2011, 469, 216–220. [CrossRef] [PubMed]
46. Capasso, M.; Devoto, M.; Hou, C.; Asgharzadeh, S.; Glessner, J.T.; Attiyeh, E.F.; Mosse, Y.P.; Kim, C.; Diskin, S.J.; Cole, K.A.; et al. Common variations in BARD1 influence susceptibility to high-risk neuroblastoma. Nat. Genet. 2009, 41, 718–723. [CrossRef] [PubMed]

47. Maris, J.M.; Mosse, Y.P.; Bradfield, J.P.; Hou, C.; Monni, S.; Scott, R.H.; Asgharzadeh, S.; Attiyeh, E.F.; Diskin, S.J.; Laudenslager, M.; et al. Chromosome 6p22 locus associated with clinically aggressive neuroblastoma. N. Engl. J. Med. 2008, 358, 2585–2593. [CrossRef] [PubMed]

48. Latorre, V.; Diskin, S.J.; Diamond, M.A.; Zhang, H.; Hakonarson, H.; Maris, J.M.; Devoto, M. Replication of neuroblastoma SNP association at the BARD1 locus in African-Americans. Cancer Epidemiol. Biomark. Prev. 2012, 21, 658–663. [CrossRef] [PubMed]

49. Zhang, R.; Zou, Y.; Zhu, J.; Zeng, X.; Yang, T.; Wang, F.; He, J.; Xia, H. The Association between GWAS-identified BARD1 Gene SNPs and Neuroblastoma Susceptibility in a Southern Chinese Population. Int. J. Med. Sci. 2016, 13, 133–138. [CrossRef] [PubMed]

50. Cimmino, F. BARD1 locus of neuroblastoma susceptibility; Università di Napoli Federico II: Naples, Italy, 2017.

51. Bosse, K.R.; Diskin, S.J.; Cole, K.A.; Wood, A.C.; Schnep, R.W.; Norris, G.; Nguyen, L.B.; Jagannathan, J.; Laquaglia, M.; Winter, C.; et al. Common variation at BARD1 results in the expression of an oncogenic isoform that influences neuroblastoma susceptibility and oncogenicity. Cancer Res. 2012, 72, 2068–2078. [CrossRef] [PubMed]

52. Capasso, M.; Calabrese, F.M.; Iolascon, A.; Mellerup, E. Combinations of genetic data in a study of neuroblastoma risk genotypes. Cancer Genet. 2014, 207, 94–97. [CrossRef] [PubMed]

53. Fu, W.; Zhu, J.; Xiong, S.W.; Jia, W.; Zhao, Z.; Zhu, S.B.; Hu, J.H.; Wang, F.H.; Xia, H.; He, J.; et al. BARD1 gene polymorphisms confer neuroblastoma susceptibility. EBioMedicine 2017, 16, 101–105. [CrossRef] [PubMed]

54. Gonzalez-Hormazabal, P.; Reyes, J.M.; Blanco, R.; Bravo, T.; Carrera, I.; Peralta, O.; Gomez, F.; Waugh, E.; Margarit, S.; Ibanez, G.; et al. The BARD1 Cys557Ser variant and risk of familial breast cancer in a South-American population. Mol. Biol. Rep. 2012, 39, 8091–8098. [CrossRef] [PubMed]

55. Jakubowska, A.; Cybulski, C.; Szymanska, A.; Huzarski, T.; Byrski, T.; Gronwald, J.; Debnik, T.; Gorski, B.; Kowalska, E.; Narod, S.A.; et al. BARD1 and breast cancer in Poland. Breast Cancer Res. Treat. 2008, 107, 119–122. [CrossRef] [PubMed]

56. Spurdle, A.B.; Marquart, L.; McGuffog, L.; Healey, S.; Sinilnikova, O.; Wan, F.; Chen, X.; Beesley, J.; Singer, C.F.; Dressler, A.C.; et al. Common genetic variation at BARD1 is not associated with breast cancer risk in BRCA1 or BRCA2 mutation carriers. Cancer Epidemiol. Biomark. Prev. 2011, 20, 1032–1038. [CrossRef] [PubMed]

57. Ding, D.P.; Zhang, Y.; Ma, W.L.; He, X.F.; Wang, W.; Yu, H.L.; Guo, Y.B.; Zheng, W.L. Lack of association between BARD1 Cys557Ser variant and breast cancer risk: A meta-analysis of 11,870 cases and 7687 controls. J. Cancer Res. Clin. Oncol. 2011, 137, 1463–1468. [CrossRef] [PubMed]

58. Johnatty, S.E.; Beesley, J.; Chen, X.; Hopper, J.L.; Southey, M.C.; Giles, G.G.; Goldgar, D.E.; Chenevix-Trench, G.; Spurdle, A.B. The BARD1 Cys557Ser polymorphism and breast cancer risk: An Australian case-control and family analysis. Breast Cancer Res. Treat. 2009, 115, 145–150. [CrossRef] [PubMed]

59. Robinson, D.R.; Wu, Y.M.; Lonigro, R.J.; Vats, P.; Cobain, E.; Everett, J.; Cao, X.; Rabban, E.; Kumar-Sinha, C.; Raymond, V.; et al. Integrative clinical genomics of metastatic cancer. Nature 2017, 548, 297–303. [CrossRef] [PubMed]

60. Carter, H.; Chen, S.; Isik, L.; Tyekucheva, S.; Velculescu, V.E.; Kinzler, K.W.; Vogelstein, B.; Karchin, R. Cancer-specific high-throughput annotation of somatic mutations: Computational prediction of driver missense mutations. Cancer Res. 2009, 69, 6660–6667. [CrossRef] [PubMed]

61. Carter, H.; Douville, C.; Stenson, P.D.; Cooper, D.N.; Karchin, R. Identifying Mendelian disease genes with the variant effect scoring tool. BMC Genom. 2013, 14 (Suppl. 3), S3. [CrossRef] [PubMed]

62. Starita, L.M.; Machida, Y.; Sankaran, S.; Elias, J.E.; Griffin, K.; Schlegel, B.P.; Gygi, S.P.; Parvin, J.D. BRCA1-dependent ubiquitination of gamma-tubulin regulates centrosome number. Mol. Cell. Biol. 2004, 24, 8457–8466. [CrossRef] [PubMed]

63. Hsu, L.C.; Doan, T.P.; White, R.L. Identification of a gamma-tubulin-binding domain in BRCA1. Cancer Res. 2001, 61, 7713–7718. [PubMed]

64. Westernmark, U.K.; Reyngold, M.; Olshen, A.B.; Baer, R.; Jasim, M.; Moynahan, M.E. BARD1 participates with BRCA1 in homology-directed repair of chromosome breaks. Mol. Cell. Biol. 2003, 23, 7926–7936. [CrossRef] [PubMed]
65. Joukov, V.; Groen, A.C.; Prokhorova, T.; Gerson, R.; White, E.; Rodriguez, A.; Walter, J.C.; Livingston, D.M. The BRCA1/BARD1 heterodimer modulates ran-dependent mitotic spindle assembly. *Cell* 2006, 127, 539–552. [CrossRef] [PubMed]

66. Zhao, W.; Manley, J.L. Deregulation of poly(A) polymerase interferes with cell growth. *Mol. Cell. Biol.* 1998, 18, 5010–5020. [CrossRef] [PubMed]

67. Kleiman, F.E.; Manley, J.L. The BARD1-CstF-50 interaction links mRNA 3′ end formation to DNA damage and tumor suppression. *Cell* 2001, 104, 743–753. [CrossRef]

68. Dechend, R.; Hirano, F.; Lehmann, K.; Heissmeyer, V.; Ansieau, S.; Wulczyn, F.G.; Scheidereit, C.; Leutz, A. The Bcl-3 oncoprotein acts as a bridging factor between NF-κB/Rel and nuclear co-regulators. *Oncogene* 1999, 18, 3316–3323. [CrossRef] [PubMed]

69. Li, M.; Yu, X. Function of BRCA1 in the DNA damage response is mediated by ADP-ribosylation. *Cancer Cell* 2013, 23, 693–704. [CrossRef] [PubMed]

70. Feki, A.; Jefford, C.E.; Berardi, P.; Wu, J.Y.; Cartier, L.; Krause, K.H.; Irminger-Finger, I. BARD1 induces apoptosis by catalysing phosphorylation of p53 by DNA-damage response kinase. *Oncogene* 2005, 24, 3726–3736. [CrossRef] [PubMed]

71. Irminger-Finger, I.; Leung, W.C.; Li, J.; Dubois-Dauphin, M.; Harb, J.; Feki, A.; Jefford, C.E.; Soriano, J.V.; Jaconi, M.; Montesano, R.; et al. Identification of BARD1 as mediator between proapoptotic stress and p53-dependent apoptosis. *Cell* 2001, 8, 1255–1266. [CrossRef]

72. Jefford, C.E.; Feki, A.; Harb, J.; Krause, K.H.; Irminger-Finger, I. Nuclear-cytoplasmic translocation of BARD1 is linked to its apoptotic activity. *Oncogene* 2004, 23, 3509–3520. [CrossRef] [PubMed]

73. Choudhury, A.D.; Xu, H.; Baer, R. Ubiquitination and proteasomal degradation of the BRCA1 tumor suppressor is regulated during cell cycle progression. *J. Biol. Chem.* 2004, 279, 33909–33918. [CrossRef] [PubMed]

74. Hayami, R.; Sato, K.; Wu, W.; Nishikawa, T.; Hiroi, J.; Ohtani-Kaneko, R.; Fukuda, M.; Ohta, T. Down-regulation of BRCA1-BARD1 ubiquitin ligase by CDK2. *Cancer Res.* 2005, 65, 6–10. [PubMed]

75. Smith, P.D.; Crossland, S.; Parker, G.; Osin, P.; Brooks, L.; Waller, J.; Philp, E.; Crompton, M.R.; Gusterson, B.A.; Allday, M.J.; et al. Novel p53 mutants selected in BRCA-associated tumours which dissociate transformation suppression from other wild-type p53 functions. *Oncogene* 1999, 18, 2451–2459. [CrossRef] [PubMed]

76. Phillips, K.A.; Nichol, K.; Ozcelik, H.; Knight, J.; Done, S.J.; Goodwin, F.J.; Andrilis, I.L. Frequency of p53 mutations in breast carcinomas from Ashkenazi Jewish carriers of BRCA1 mutations. *J. Natl. Cancer Inst.* 1999, 91, 469–473. [CrossRef] [PubMed]

77. Li, L.; Ryser, S.; Dizin, E.; Pils, D.; Krainer, M.; Jefford, C.E.; Bertoni, F.; Zeillinger, R.; Irminger-Finger, I. Oncogenic BARD1 isoforms expressed in gynecological cancers. *Cancer Res.* 2007, 67, 11876–11885. [CrossRef] [PubMed]

78. Zhang, Y.Q.; Bianco, A.; Malkinson, A.M.; Leoni, V.P.; Frau, G.; De Rosa, N.; Andre, P.A.; Versace, R.; Boulvain, M.; Laurent, G.J.; et al. BARD1: An independent predictor of survival in non-small cell lung cancer. *Int. J. Cancer* 2012, 131, 83–94. [CrossRef] [PubMed]

79. Feki, A.; Jefford, C.E.; Durand, P.; Harb, J.; Lucas, H.; Krause, K.H.; Irminger-Finger, I. BARD1 expression during spermatogenesis is associated with apoptosis and hormonally regulated. *Biol. Reprod.* 2004, 71, 1614–1624. [CrossRef] [PubMed]

80. Tsuzuki, M.; Wu, W.; Nishikawa, H.; Hayami, R.; Oyake, D.; Yabuki, Y.; Fukuda, M.; Ohta, T. A truncated splice variant of human BARD1 that lacks the RING finger and ankyrin repeats. *Cancer Lett.* 2006, 233, 108–116. [CrossRef] [PubMed]

81. Li, L.; Cohen, M.; Wu, J.; Sow, M.H.; Nikolic, B.; Bischof, P.; Irminger-Finger, I. Identification of BARD1 splice-isomers involved in human trophoblast invasion. *Int. J. Biochem. Cell Biol.* 2007, 39, 1659–1672. [CrossRef] [PubMed]

82. Tembe, V.; Henderson, B.R. BARD1 translocation to mitochondria correlates with Bax oligomerization, loss of mitochondrial membrane potential and apoptosis. *J. Biol. Chem.* 2007, 282, 20513–20522. [CrossRef] [PubMed]

83. Dizin, E.; Irminger-Finger, I. Negative feedback loop of BRCA1-BARD1 ubiquitin ligase on estrogen receptor α stability and activity antagonized by cancer-associated isoform of BARD1. *Int. J. Biochem. Cell Biol.* 2010, 42, 693–700. [CrossRef] [PubMed]
84. Pilyugin, M.; Andre, P.A.; Ratajska, M.; Kuzniacka, A.; Limon, J.; Tournier, B.B.; Colas, J.; Laurent, G.; Irminger-Finger, I. Antagonizing functions of BARD1 and its alternatively spliced variant BARD1δ in telomere stability. Oncotarget 2017, 8, 9339–9353. [CrossRef] [PubMed]

85. Lepore, I.; Dell’Aversana, C.; Pilyugin, M.; Conte, M.; Nebbioso, A.; De Bellis, F.; Tambaro, F.P.; Izzo, T.; Garcia-Manero, G.; Ferrara, F.; et al. HDAC inhibitors repress BARD1 isoform expression in acute myeloid leukemia cells via activation of miR-19a and/or b. PLoS ONE 2013, 8, e83018. [CrossRef] [PubMed]

86. Capasso, M.; Diskin, S.J. Genetics and genomics of neuroblastoma. Cancer Treat. Res. 2010, 155, 65–84. [PubMed]

87. Bryant, H.E.; Schultz, N.; Thomas, H.D.; Parker, K.M.; Flower, D.; Lopez, E.; Kyle, S.; Meuth, M.; Curtin, N.J.; Hellday, T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005, 434, 913–917. [CrossRef] [PubMed]

88. Ledermann, J.; Harter, P.; Gourley, C.; Friedlander, M.; Vergote, I.; Rustin, G.; Scott, C.L.; Meier, W.; Shapira-Frommer, R.; Safra, T.; et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: A preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol. 2014, 15, 852–861. [CrossRef]

89. Lord, C.J.; Ashworth, A. Mechanisms of resistance to therapies targeting BRCA-mutant cancers. Nat. Med. 2013, 19, 1381–1388. [CrossRef] [PubMed]

90. Ozden, O.; Bishehsari, F.; Bauer, J.; Park, S.H.; Jana, A.; Baik, S.H.; Sporn, J.C.; Staudacher, J.J.; Yazici, C.; Krett, N.; et al. Expression of an oncogenic BARD1 splice variant impairs homologous recombination and predicts response to PARP-1 inhibitor therapy in colon cancer. Sci. Rep. 2016, 6, 26273. [CrossRef] [PubMed]

91. Pilyugin, M.; Descloux, P.; Andre, P.A.; Laszlo, V.; Dome, B.; Hegedus, B.; Sardy, S.; Janes, S.; Bianco, A.; Laurent, G.; et al. BARD1 serum autoantibodies for the detection of lung cancer. PLoS ONE 2017, 12, e0182356. [CrossRef] [PubMed]