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BARD1

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BARD1 was originally identified as a protein interacting with BRCA1, the breast cancer predisposition gene product. BARD1, like BRCA1, has an amino-terminal RING-finger domain and carboxy-terminal BRCT domains. In addition, BARD1 has three ankyrin repeats adjacent to the BRCT domains. BARD1 and BRCA1 form a stable heterodimer via their RING-finger domains. BRCA1, like many RING-finger proteins, has E3 ubiquitin ligase activity, which is amplified when in association with BARD1. By contrast, BARD1 alone has no such activity. The binding of BARD1 to BRCA1 stabilizes BRCA1 and, to some extent, BARD1. BARD1 and BRCA1 are co-expressed in most proliferating tissues and are localized to the nucleus. Based mostly on its ubiquitin ligase activity, the BARD1/BRCA1 complex has functions in DNA repair, transcriptional regulation, chromatin condensation, cell-cycle regulation, mitotic spindle formation and cytokinesis. BARD1 is highly conserved, having orthologs in many species. In mice, BARD1 has essential functions during development, and *Bard1*-null mice, like *Brca1*-null mice, die between embryonic days 7 and 8, suggesting that the defect is caused by the lack of a functional BARD1/BRCA1 heterodimer. Whereas only a few somatic and germline mutations of *BARD1* have been found associated with breast, ovarian and endometrial cancer, nine commonly found alleles of *BARD1* that contain single nucleotide polymorphisms have been found to be significantly associated with childhood neuroblastoma. In breast and ovarian cancers, truncated forms of BARD1 derived by differential RNA splicing are highly upregulated and are localized to the cytoplasm. The expression of these BARD1 isoforms in breast and ovarian cancer is correlated with a poor prognosis.

KEYWORDS
Bard1; BARD1; BRCA1-associated RING domain 1; Breast cancer 1 associated RING domain protein 1

IDENTIFIERS
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PROTEIN FUNCTION
The BARD1 protein is a binding partner of the breast cancer predisposition gene product BRCA1 (Wu *et al*. 1996). The binding of BARD1 to BRCA1 is required for BRCA1 stability and nuclear localization (Fabbro *et al*. 2004b; Fabbro *et al*. 2002; Joukov *et al*. 2001; Rodriguez *et al*. 2004). The BARD1/BRCA1 heterodimer has E3 ubiquitin ligase activity, and many functions of BARD1 and BRCA1 are based on this ubiquitin ligase activity (Baer and Ludwig 2002; Hashizume *et al*. 2001; Morris *et al*. 2009). The RING-finger domain of BRCA1, which comprises the amino-terminal 300 amino acids of BRCA1, is sufficient for ubiquitin ligase activity, but this activity increases significantly when BRCA1 is bound to BARD1 or to the RING-finger domain of BARD1 (Hashizume *et al*. 2001; Xia *et al*. 2003). Ubiquitin ligase activity has not been reported for BARD1 or its RING-finger domain.

BARD1 co-purifies with the BRCA1/RNA polymerase II (RNA Pol II) complex, and it is thought that the ubiquitin ligase BARD1/BRCA1 controls cell-cycle progression by targeting proteins of the stalled RNA Pol II complex (Chiba and Parvin 2002; Kleiman *et al*. 2005). The binding of BARD1 to CstF-50, which is induced by DNA damage after exposure to hydroxyurea or ultraviolet radiation, is linked to degradation of the IIO subspecies of RNA Pol II by BARD1/BRCA1. This process initiates or facilitates DNA repair pathways by inhibiting the RNA processing machinery (Kleiman *et al*. 2005; Kleiman and Manley 2001; Kleiman and Manley 1999).

Furthermore, disruption to the BARD1/BRCA1 heterodimer has been described to inhibit double-strand-break repair (Stark *et al*. 2004). Considerable attention has been drawn to the role of the heterodimer in homologous recombination (Ciccia and Elledge 2010; Laufer *et al*. 2007; Sobhian *et al*. 2007; Wang and Elledge 2007). Interestingly, ubiquitin-ligase-deficient BRCA1 is functional in double-strand-break repair pathways, suggesting that ubiquitin ligase activity is not required for this function (Reid *et al*. 2008).

A role for BARD1/BRCA1 in cell-cycle control is imposed by the ubiquitylation of γ-tubulin, which is involved in centrosome duplication, an important anaphase checkpoint (Starita and Parvin 2006; Starita *et al*. 2005). BARD1/BRCA1 monoubiquitylates the histone H2AX, indicating that it also has a role in chromatin modification (Starita and Parvin 2006).

BARD1/BRCA1 has been proposed to have a role in spindle formation, through binding to spindle-pole proteins in a RAN-dependent manner (Joukov *et al*. 2006). Other regulatory roles of BARD1/ BRCA1 involve the timed degradation of the mitotic kinase aurora kinase B during mitosis and a function in regulating exit from mitosis (Ryser *et al*. 2009).

Furthermore, BARD1/BRCA1 has been shown to ubiquitylate estrogen receptor-α *in vitro* (Eakin *et al*. 2007), which might provide an explanation for the association of *BRCA1* and *BARD1* mutations with cancers of hormone-dependent tissues. The role of BRCA1 and BARD1 in estrogen receptor-α degradation has been confirmed by depletion and overexpression studies (Dizin and Irminger-Finger 2010).

BARD1 regulation and function is likely to be independent of BRCA1, on the basis of the following five findings. First, not all BARD1 is bound to BRCA1 in the cell (Chiba and Parvin 2002; Chiba and Parvin 2001).

Second, apart from the RING-finger and BRCT domains, which are common to both BRCA1 and BARD1, BARD1 is dissimilar

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in that it is more highly conserved than BRCA1 and also contains ankyrin motifs. The ankyrin repeats and BRCT domains might provide a functional hub for protein interactions, for example for binding of the tumor-suppressor protein p53 (Jefford *et al*. 2004) or of CstF-50 (Edwards *et al*. 2008; Kleiman and Manley 2001), although interaction with CstF-50 can be linked to the DNA repair functions of BARD1/BRCA1 (Kleiman *et al*. 2005; Kleiman and Manley 2001). Recently, p53 was reported to inhibit the 3′ processing of messenger RNA, through its interaction with the CstF1/BARD1 complex (Nazeer *et al*. 2011). Specifically, the linker region of BARD1 between the ankyrin repeats and the BRCT domains is necessary and sufficient for p53 and CstF-50 binding and has been found to contain mutations that are associated with breast and ovarian cancer: Cys557Ser and Gln564His. The Gln564His mutation disrupts CstF-50 binding (Kleiman and Manley 2001) and BARD1-mediated apoptosis (Irminger-Finger *et al*. 2001; Sauer and Andrulis 2005). A genetic link is suspected between the Cys557Ser mutation and *BRCA2* mutations, increasing the risk of carrying a double mutation (Stacey *et al*. 2006).

Third, the interactions of BARD1 with certain proteins, as mentioned in the second point above, have been shown not to require the BRCA1-interaction domain of BARD1.

Fourth, BARD1 isoforms are expressed in gynecological cancers and are associated with a poor prognosis (Li *et al*. 2007; Wu *et al*. 2006). Single nucleotide polymorphisms (SNPs) that have strong linkage disequilibrium and are associated with aggressive neuroblastoma have been found in the 5′-untranslated region and introns 1, 3 and 4 of *BARD1* (Capasso *et al*. 2009), suggesting that these mutations might affect the splicing of the corresponding exons.

Last, BARD1, but not BRCA1, has been identified in several protein/protein interaction screens. For example, BARD1 was identified as a substrate for the ubiquitin ligase APC/C (the anaphase-promoting complex/cyclosome), which selectively targets BARD1 for degradation (Song and Rape 2010). This finding shows that the degradation of BARD1 can be regulated independently from that of BRCA1.

The binding of BARD1 to the nuclear factor-κB (NF-κB) subunit BCL-3 might be involved in transcriptional regulation (Dechend *et al*. 1999), because this interaction modulates the transcriptional activity of NF-κB.

BARD1 also functions in the apoptotic pathway, and several studies suggest that BARD1 binds to, and stabilizes, p53 (Fabbro *et al*. 2004a; Fabbro *et al*. 2004b; Feki *et al*. 2005; Irminger-Finger *et al*. 2001; Jefford *et al*. 2004; Sauer and Andrulis 2005). In particular, BARD1 has been suggested to be required for phosphorylation of p53 on Ser 37 in response to DNA damage, and the induction of apoptosis in cells with wild-type p53 is inhibited in the absence of BARD1 (Fabbro *et al*. 2004a; Feki *et al*. 2005). Interestingly, BARD1 is localized to mitochondria, where p53 can also be found (Tembe and Henderson 2007). Thus, BARD1 and p53 might have a function in regulation of the potential of the mitochondrial membrane in apoptosis.

REGULATION OF ACTIVITY

The regulation of the E3 ubiquitin ligase activity of BARD1/BRCA1 has not been investigated directly. One regulatory factor, presumably, is protein stability, which is conferred on both proteins through heterodimerization. Thus, the regulation of BARD1/BRCA1 ubiquitin ligase activity might depend on the transcriptional and post-translational regulation of each partner protein, in particular by phosphorylation and ubiquitylation (Choudhury *et al*. 2005; Choudhury *et al*. 2004; Mallery *et al*. 2002). Another reported mechanism of regulation of the ubiquitin ligase activity is SUMOylation (Galanty *et al*. 2009; Morris *et al*. 2009).

Downregulation of BARD1/BRCA1 ubiquitin ligase activity, however, has also been reported, through phosphorylation mediated by cyclin-dependent kinases (Hayami *et al*. 2005). In addition, inactivation of BARD1/BRCA1 ubiquitin ligase activity has been observed through the binding of UBXN1 to auto-ubiquitylated BRCA1, which regulates the enzymatic function of BRCA1 (Wu-Baer *et al*. 2010).

Co-expression of BARD1 and BRCA1 is found in most, but not all, hormonally regulated tissues (Irminger-Finger *et al*. 1998). The transcription of *BARD1* is regulated by hormones, oxidative stress and cell-cycle-specific transcription factors (Ayi *et al*. 1998; Feki *et al*. 2004; Irminger-Finger *et al*. 2001; Irminger-Finger *et al*. 1998; Li *et al*. 2007a).

The interaction of BARD1/BRCA1 with DNA repair proteins is induced by genotoxic stress (Greenberg *et al*. 2006). Ultraviolet radiation and hydroxyurea induce the translocation of BARD1 and BRCA1 to sites of DNA repair. DNA damage signaling involves the protein kinase ATM, and C-terminal epitopes of BARD1 are targets of phosphorylation by ATM (Kim *et al*. 2006).

The apoptotic activity of BARD1 depends on its expression being upregulated, whereas upregulated expression of BRCA1 reduces BARD1-dependent apoptosis (Feki *et al*. 2005; Henderson 2005; Irminger-Finger *et al*. 2001; Jefford *et al*. 2004; Rodriguez *et al*. 2004; Schüchner *et al*. 2005). BARD1 is localized to mitochondria, where p53 can also be found (Tembe and Henderson 2007). Thus, BARD1 and p53 might function to regulate the potential of the mitochondrial membrane in apoptosis.

INTERACTIONS

The major protein binding partner of BARD1 is BRCA1 (Wu *et al*. 1996). In addition to proteins that associate with BARD1/BRCA1, BARD1 binds directly to the polyadenylation factor CstF-50 (Kleiman and Manley 1999). This interaction might be functionally linked to the DNA repair functions of BARD1/BRCA1 (Kleiman *et al*. 2005; Kleiman and Manley 2001). In addition, BARD1 enhances p53 phosphorylation, which is required for the induction of apoptosis, by binding to p53 and DNA-dependent protein kinase (DNA-PK) (Fabbro *et al*. 2004; Feki *et al*. 2005).

Another protein binding partner of BARD1 is the NF-κB subunit BCL-3 (Dechend *et al*. 1999). Interestingly, the C-terminal domain of BARD1 has also been found to interact with the Ewing sarcoma oncogene product (Spahn *et al*. 2002). Specific interaction of BARD1, but not BRCA1, with the human papilloma virus protein E6 has also been reported (Yim *et al*. 2007).

The number of known BARD-1-interacting proteins increased with the identification of numerous target proteins of BARD1/BRCA1 ubiquitin ligase activity, including γ-tubulin (Starita and Parvin 2006), nucleophosmin (Sato et al. 2004), the

histone H2AX and the GTPase RAN (Joukov *et al*. 2006), and with the finding that BARD1 also binds directly to targets of the BARD1/BRCA1 ubiquitin ligase, in particular to aurora B, for whose degradation it is required (Ryser *et al*. 2009). It was also recently shown that BARD1 binds to estrogen receptor-α and is required for its degradation (Eakin et al. 2007; Dizin *et al*. 2010).

PHENOTYPES

Depletion of BARD1 in mice leads to embryonic lethality at day 7–8 (McCarthy *et al*. 2003). The phenotype of the *Bard1* knockout mouse is indistinguishable from the phenotype of the *Brca1* knockout, presumably because of the stabilizing effect of BARD1 on BRCA1. In *ex vivo* cultures of *Bard1*$^{-/-}$ embryos, defects of outgrowth of the inner cell mass are observed (McCarthy *et al*. 2003).

Conditional mutants of *Bard1* and *Brca1* also show a similar phenotype of tumor formation in mammary tissues (Shakya *et al*. 2008), which suggests that the essential tumor-suppressor functions of BARD1 and BRCA1 are maintained by the BARD1/BRCA1 heterodimer. Interestingly, ubiquitin-ligase-deficient BRCA1 still functions in double-strand-break repair pathways, suggesting that E3 ubiquitin ligase activity is not required for this repair function (Reid *et al*. 2008).

Studies in *Arabidopsis thaliana* have shown that BARD1 is involved in DNA repair. In addition, an insertion mutation that disrupted the BRCT domain, resulting in deletion of the C-terminal part of the molecule, had marked effects on meristem formation (Han *et al*. 2008). This finding suggests that the C-terminal domain has essential functions. By contrast, insertion mutations in introns 1 or 2, resulting in deletion of the N-terminal RING-finger domain, did not lead to an observable change in phenotype (Han *et al*. 2008).

Repression of *BARD1 in vitro* leads to aberrant cell-cycle progression, loss of contact inhibition of cell growth, loss of epithelial cell polarity and genetic instability (Irminger-Finger *et al*. 1998). Selective repression of either BARD1 or BARD1 isoforms that lack the N-terminal RING finger showed that the C terminus of BARD1 is required for cytokinesis and interacts with aurora B and BRCA2, as well as that BARD1 repression results in inhibition of these steps and a block in cytokinesis (Ryser *et al*. 2009).

Only a few somatic and germline mutations of *BARD1* have been associated with cancers of the breast, ovary, uterus and a small number of hereditary cancers (Ghimenti *et al*. 2002; Huo *et al*. 2007; Karppinen *et al*. 2006; Karppinen *et al*. 2004; Stacey *et al*. 2006; Thai *et al*. 1998; Vahteristo *et al*. 2006). Most of these mutations lead to alterations in the C-terminal part of the protein and do not involve the RING finger and hence the ability of BARD1 to bind to BRCA1. Notably, truncated or deletion-bearing forms of BARD1 that are derived from differential splicing are highly upregulated and localized to the cytoplasm in breast and ovarian cancer, and these isoforms correlate with markers of poor prognosis (Li *et al*. 2007a; Wu *et al*. 2006). The most frequently found splice variants—which have also been identified in normal human cytotrophoblasts (Li *et al*. 2007b), peripheral blood cells (Lombardi *et al*. 2007) and cell lines (Feki *et al*. 2005; Tsuzuki *et al*. 2005)—are derived from the skipping of exons 2, 3 and 4. Thus, these variants lack all or part of the RING-finger domain, and some also lack the ankyrin repeats; all forms retain the most C-terminal exon.

Furthermore, nine SNP alleles of *BARD1* have been found to be significantly associated with childhood neuroblastomas of poor prognosis (Capasso *et al*. 2009). These SNPs are located in the 5′ upstream region of *BARD1* (which is composed of 11 exons) and in introns 1, 3 and 4, and they show strong linkage disequilibrium.

MAJOR SITES OF EXPRESSION

BARD1 is expressed in most proliferating cells and organs (Irminger-Finger *et al*. 1998). Maximal expression has been found in the testes and spleen, with no or low expression in the central nervous system, of healthy mice (Ayi *et al*. 1998; Feki *et al*. 2004; Irminger-Finger *et al*. 2001; Irminger-Finger *et al*. 1998). High expression has also been found in granulosa cells and at specific stages of oogenesis (Gasca *et al*. 2007).

Highly upregulated cytoplasmic expression has been reported in mice with hypoxia-induced brain damage (Irminger-Finger *et al*. 2001), in human cytotrophoblasts (Li *et al*. 2007a) and in a large number of human cancers of epithelial origin (Gautier *et al*. 2000; Li *et al*. 2007b; Wu *et al*. 2006). Differential expression of *BARD1* mRNA between normal and malignant breast tissue has also been reported (Reinholz *et al*. 2004). Notably, *BARD1* SNPs are associated with neuroblastomas of poor outcome, suggesting that *BARD1* expression might also have a role in this type of cancer (Capasso *et al*. 2009).

SPLICE VARIANTS

Several splice variants of BARD1 have been reported. During spermatogenesis, BARD1β, which lacks exons 2 and 3, is expressed in spermatocytes, whereas spermatogonia express full-length BARD1 (Feki *et al*. 2004). In HeLa cells, BARD1δ, an isoform that lacks exons 2 to 6, has been found (Tsuzuki *et al*. 2006). This isoform has also been observed in a rat ovarian cancer cell line (Feki *et al*. 2005). BARD1δ localizes to mitochondria but, unlike wild-type BARD1, does not contribute to mitochondria-mediated apoptosis (Tembe and Henderson 2007).

A complex pattern of splice variant expression is found in human cytotrophoblasts and in breast and ovarian cancer (Li *et al*. 2007a; Li *et al*. 2007b; Lombardi *et al*. 2007; Wu *et al*. 2006). In these cases, the isoforms BARD1α (which lacks exon 2), BARD1β (which lacks exons 2 and 3), BARD1γ (which lacks exon 4), BARD1δ (as in HeLa cells), BARD1φ (which lacks exons 3 to 6), BARD1ε (which lacks exons 4 to 9) and BARD1η (which lacks exons 2 to 9) are expressed at the RNA level. All of these, with the exception of BARD1γ, could be translated into proteins. BARD1β binds to the protein kinase aurora B and antagonizes its degradation, which is induced through ubiquitylation by BARD1/BRCA1 (Ryser *et al*. 2009).

REGULATION OF CONCENTRATION

The transcription of *BARD1* is controlled by hormones, as has been shown in spermatogenesis and oogenesis (Ayi *et al*. 1998; Feki *et al*. 2004; Irminger-Finger *et al*. 1998). Specifically, estrogen concentration might be tightly linked to *BARD1* expression, because the stability of the estrogen receptor might be controlled by the BARD1/BRCA1 ubiquitin ligase (Dizin and Irminger-Finger 2010; Eakin *et al*. 2007) and because *BARD1* expression is regulated by estrogen (Creekmore *et al*. 2007; Gasca *et al*. 2007).

BARD1 expression is also regulated by cell-cycle progression, through the actions of the transcription factor E2F (Ren *et al*. 2002). BARD1 is phosphorylated during mitosis (Choudhury *et*

al. 2005; Hayami *et al*. 2005). In addition, the concentration of BARD1 protein has been reported to increase in mitosis (Jefford *et al*. 2004; Ryser *et al*. 2009). Together, these findings suggest that the phosphorylation of BARD1 could increase its stability.

BARD1 is upregulated following various types of cellular stress. Induction of mRNA and protein expression is observed in response to genotoxic agents, such as ultraviolet radiation, chemotherapeutic drugs and hypoxia (Irminger-Finger *et al*. 2001; Li *et al*. 2007a). Hypoxia induces *BARD1* expression at the mRNA and protein levels, both *in vitro* and *in vivo*. Hormonal regulation and hypoxia might also have a role in the upregulation of *BARD1* expression in various cancers (Li *et al*. 2007b; Wu *et al*. 2006).

The selective degradation of BARD1, as a target of the ubiquitin ligase APC/C, has been reported recently (Song and Rape 2010).

ANTIBODIES

Several of the reports mentioned above used commercially available antibodies directed against exon 4 sequences of BARD1 (Bethyl Laboratories). Santa Cruz Biotechnology provides antibodies against the N and C terminus of BARD1 (N-19 and C-20, respectively), as well as a polyclonal antibody against the N-terminal 300 amino acids (H-300).

A monoclonal antibody that detects the BARD1 RING-finger domain was originally reported by R. Baer's research group (Jin *et al*. 1997; Yu and Baer 2000) and has since been used by many other researchers. An antibody directed against the RING-finger domain of *Xenopus laevis* bard1 is also in use (Joukov *et al*. 2001). Polyclonal rabbit antibodies directed against epitopes adjacent to the RING finger (Feki *et al*. 2005; Feki *et al*. 2004; Irminger-Finger *et al*. 2001; Irminger-Finger *et al*. 1998; Jefford *et al*. 2004), as well as to an alternative open reading frame of isoform BARD1β (Ryser *et al*. 2009), have also been described.

Table 1: Functional States

STATE DESCRIPTION	LOCATION	REFERENCES
BARD1	nucleus	Fabbro M *et al.* 2004; Fabbro M *et al.* 2004; Feki A *et al.* 2005
BARD1-T702, T722P2	Unknown	Kim HS *et al.* 2006
BARD1-P7	Unknown	Choudhury AD *et al.* 2005
BARD1-Ub	Unknown	Chen A *et al.* 2002
BARD1-P2-Ub/BRCA1-P-Ub	Unknown	Chen A *et al.* 2002; Mallery DL *et al.* 2002
BARD1/BRCA1/CstF50	Unknown	Edwards RA *et al.* 2008; Kleiman FE and Manley JL 2001; Kleiman FE and Manley JL 1999
BARD1/Bcl3	Unknown	Dechend R *et al.* 1999
BARD1/EWS	Unknown	Spahn L *et al.* 2002
BARD1β/AurB	Unknown	Ryser S *et al.* 2009

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This molecule exists in 9 states , has 8 transitions between these states and has 9 enzyme functions.(Please zoom in the pdf file to view details.)