Significance of the RING domain of the BRCA1 gene - Review of Missense Mutations

Esha Desai

Abstract

Breast cancer occurs around the world, and 2022 data reveal 2,308,897 new breast cancer cases accounted for 11.6% of all new cancer cases (Bray et al., 2024). Breast cancer susceptibility gene 1 (BRCA1) is one of the most common tumor suppressor genes and is detected in at least 5% of patients with breast cancer (Morris et al., 2006). BRCA1 gene mutations are prevalent and found in 35% of the hereditary breast cancer cases. Out of the three domains of the BRCA1 gene, the mutations in the RING domain near the N terminus are reviewed here. Out of the 27 mutations indicated in the RING domain, 14 missense mutations identified with a pathogenic outcome were selected for this review (Gracia et al., 2024). A missense mutation replaces the nucleotide in the gene that affects the protein folding when that gene is expressed, affecting the protein function. The BRCA1 protein forms a heterodimer with the BARD1 protein to perform E3 ligase activity. The RING region of BRCA1 is crucial for heterodimer formation. Suppose a mutation were to occur in the RING region, it might interfere with the two proteins binding to form a heterodimer, thus affecting E3 ligase activity that is crucial for gene regulation through the ubiquitination process. Missense mutations have been shown to have a pathogenic outcome and are therefore studied in detail.

Introduction

The BRCA1 tumor suppressor gene codes for the BRCA1 protein that repairs damaged DNA, regulates the cell cycle, and repairs damaged genes. This is because the BRCA1 Protein is a catalyst for the ubiquitination of a multitude of proteins, which are necessary to keep genomic stability of proteins like cell cycle regulators, such as Cyclin B (Morris et al., 2006). A mutation to the BRCA1 gene can lead to breast and ovarian cancer if the BRCA1 protein is not able to interact with other proteins to repair and regulate the cell. The BRCA1 gene is located on chromosome 17q21, and 24 exons make up the BRCA1 protein (Gorodetska et al., 2019). The BRCA1 gene has a conserved zinc-binding RING Domain located near the N terminus. The N-terminus Really Interesting New Gene (RING) region has E3 ligase activity, which allows the BRCA1 protein to bind with other proteins, such as BARD1 to form a heterodimer. Additionally, there are two BRCT domains located near the C Terminus. The central part of the BRCA1 gene contains two nuclear localization signals and a coiled-coil domain. There are many types of mutations identified in the RING region. Missense mutations have been shown to have a pathogenic outcome and are therefore studied in detail. Due to a change in the single nucleotide, a missense mutation leads to the formation of a new codon that codes for a new amino acid. Thus, change in the amino acid at a particular position in the BRCA1 protein leads to loss of function activity. This paper reviews the effects of missense mutations in the N-terminal RING domain of the BRCA1 gene.

BRCA1 gene

The BReast CAncer Gene 1, also known as the BRCA1 gene, is important for a multitude of reasons. It is an essential tumor suppressor as mutations to this gene significantly increase the

risk of breast and ovarian cancer (Morris et al., 2006). The BRCA1 gene suppresses tumors by repairing damaged genes, regulating the cell cycle, and other processes to make sure that malignant cells are not produced. The BRCA1 gene codes for a 1863 amino acid protein (Figure 1). BRCA1 protein has a conserved zinc-binding RING domain located near the N terminus. The N terminus RING region has E3 ligase activity which allows the BRCA1 protein to bind with other proteins such as BARD1 to form a heterodimer. Additionally, there are two BRCT domains located near the C Terminus. The central part of the BRCA1 gene has two nuclear localization signals and a coiled coil domain. The BRCA1 C-terminus domain (BRCT) is associated with repairing damaged DNA response and phosphorylating interacting proteins (Morris et al., 2006). The N-terminus RING domain is responsible for E3 ligase activity because of a heterodimer formed with the BRCA1 protein and other associated proteins such as the RING region of BARD1. This is because the RING region has a CH3CH4 zinc finger also known as the RING finger is a motif found in many different proteins. The RING finger motif is a series of conserved cysteine and histidine residues: C-X2-C-X(9-12)-C-X2-H-X2-H-X2-C-X2-H.

C = Cystine

H = Histidine

X = Any Amino Acid

This zinc finger motif uses the C3HC4 amino acid sequence which could bind two zinc ions that contribute to the protein's stability and function. The RING finger binds two Zinc ions into a "cross brace" arrangement creating a small distance between the two zinc binding sites. This is important in ubiquitination as it helps the BRCA1 gene bind to other proteins to become a E3 ubiquitin ligase.

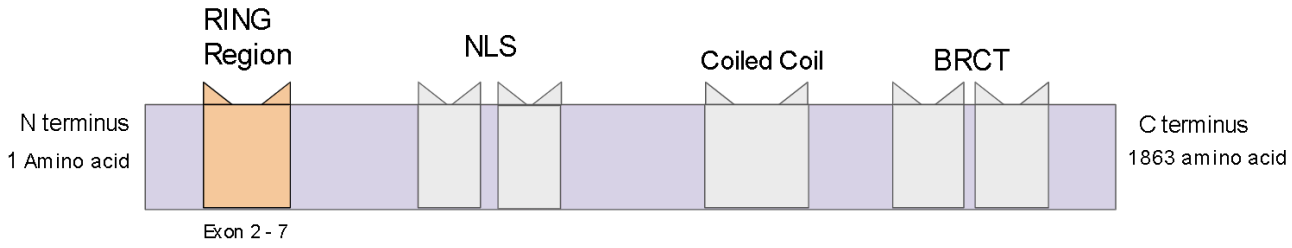

Figure 1: The BRCA1 Gene is composed of 24 exons and three main domains. Furthermore, the BRCA1 Gene has a RING Domain located at the N Terminus, two BRCT domains near the C terminus, and a coiled coil domain which codes for the BRCA1 protein (Morris et al., 2006).

Ubiquitination

Ubiquitin is a protein made up of 76 amino acids that can activate other proteins through the process of ubiquitin attachment. Ubiquitin can be found in almost any cellular tissue in the body and is soluble in water because it has a hydrophilic exterior and a hydrophobic interior. Since it is Hydrophilic, it can pass through the cell easily. Ubiquitin is a protein with many regulatory functions as it can create post-translational modifications to other proteins, known as

ubiquitination. Adding a single ubiquitin to the target protein is monoubiquitylation; attaching multiple ubiquitin molecules is polyubiquitination (Q. Yang et al., 2021). Ubiquitination is the process of ubiquitin being conjugated to the protein substrate in a three-step cascade mechanism.

1. E1 is a ubiquitin-activating enzyme that helps activate ubiquitin protein through an ATP-dependent reaction. A ubiquitin carrier enzyme can carry activated ubiquitin.
2. Activated ubiquitin attaches to the E2 enzyme (ubiquitin carrier enzyme) and is transported to ubiquitin protein ligase, E3.
3. The E3 ligase then transfers the activated ubiquitin onto the target protein.

The BRCA1 protein forms a heterodimer by binding to BARD1 and becoming an E3 ligase (Brzovic et al., 2001) (Figure 2). This happens because the RING domains on each of the proteins bind together to form a heterodimer that can function as E3 ligase (Iii et al., 2023). The Zinc finger motif in both the RING domains play an important role as the zinc ions help bind both of the RING domains together. If that were disrupted, the BRCA1 and BARD proteins would not be able to form a heterodimer. The newly formed heterodimer is the E3 ligase enzyme and brings the ubiquitin to the target protein. The E3 ligase function of the RING domain is important for maintaining the genome's integrity and transcriptional regulation (Ruffner et al., 2001).

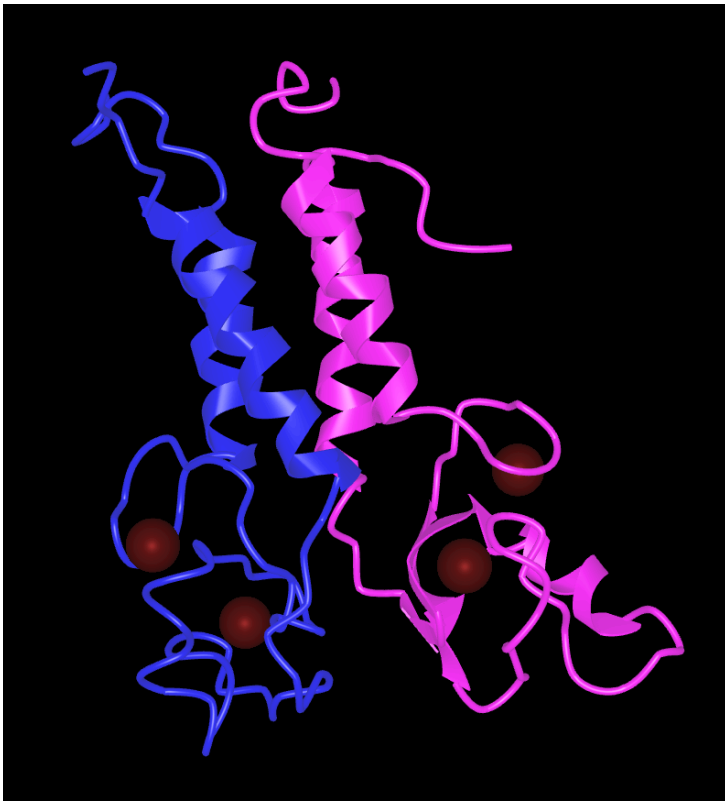

Figure 2: PDB ID 1JM7: Solution structure of the BRCA1/BARD1 (blue and pink ribbons) RING-domain heterodimer- *using NCBI tool iCn3D. The BRCA1/BARD1 Heterodimer forms an E3 ligase that is essential for Ubiquitination. This is caused when Zinc Ions (red balls) from both proteins' RING Regions attach to each other forming the Heterodimer (Gorodetska et al., 2019).*

The function of this heterodimer is to bring ubiquitin to its target protein and is the final step of the Ubiquitination process.
https://www.ncbi.nlm.nih.gov/Structure/icn3d/share2.html?2b44f21c00b0b9126b5b2f5f39df36be

In the RING domain there are 27 mutations that are from the Clinvar database. Out of those 27 mutations, they were categorized as pathogenic, uncertain, benign and only the pathogenic and likely pathogenic were reviewed. These 14 mutations are tabulated below.

Table 1: 14 missense mutations in the RING domain of BRCA1

No.	Type of mutation	Location of mutation	Residual Codon	Mutated Codon	Protein Variant	Effect of the Mutation	Accession ID	References
1.	Missense	c.53T>A	ATG	AAG	M18K	Replacement of the hydrophobic amino acid methionine (M) by the polar amino acid lysine (K) causing an altered protein.	VCV000055559	(Gracia et al., 2024' Machackova et al., 2001)
2.	Missense	c.190T>G	TGT	GGT	C64G	Switching one amino acid, a new 5' splice site is created in exon 5 and disrupts	VCV000017660	(Gracia et al., 2024' Y. Yang et al., 2003)

						the old splice site.		
3.	Missense	c.53T>C	ATG	ACG	M18T	Gene repair mechanism was impaired.	VCV000037664	(Gracia et al., 2024' Ruffner et al., 2001)
4.	Missense	c.211A>G	AGG	GGG	R71G	22 base pairs on exon 5 were deleted introducing a stop codon.	VCV000017693	(Gracia et al., 2024; Ruffner et al., 2001' Y. Yang et al., 2003)
5.	Missense	c.131G>T	TGC	TTC	C44F	C3HC4 RING finger canonical residue that is essential for binding zinc ions to keep the RING Finger structure together.	VCV000054200	(Abkevich et al., 2004' Gracia et al., 2024.)
6.	Missense	c.70T>C	TGT	CGT	C24R	Zinc binding is affected, affecting the ubiquitinati	VCV000055674	(Abkevich et al., 2004' Gracia et al., 2024)

						on process.		
7.	Missense	c.181T>G	TGT	GGT	C61G	E3 ubiquitin ligase activity stopped.	VCV000017661	(Gracia et al., 2024' Ruffner et al., 2001.)
8.	Missense	c.110C>G	ACA	AGA	T37R	E2 and E3 ligase activities are stopped.	VCV000054132	(Gracia et al., 2024' Ruffner et al., 2001.)
9.	Missense	c.139T>G	TGC	GGC	C47G	impairs E2 binding which stops E3 ligase activity.	VCV000054242	(Abkevich et al., 2004' Gracia et al., 2024; Ruffner et al., 2001.)
10.	Missense	c.115T>A	TGT	AGT	C39S	No E3 ligase activity	VCV000054151	(Abkevich et al., 2004' Gracia et al., 2024.)
11.	Missense	c.116G>A	TGT	TAT	C39Y	No E3 ligase activity	VCV00003739	(Abkevich et al., 2004' Gracia et al., 2024.)

12.	Missense	c.191G>A	TGT	TAT	C64Y	No E3 ligase activity	VCV000054400	(Abkevich et al., 2004' Gracia et al., 2024.)
13.	Missense	c.122A>G	CAC	CGC	H41R	No E3 ligase activity	VCV000054166	(Abkevich et al., 2004' Gracia et al., 2024.)
14.	Missense	c.190T>C	TGT	CGT	C64R	No E3 ligase activity	VCV000054394	(Abkevich et al., 2004' Gracia et al., 2024.)

Methods

I mainly used the PubMed database to search for information on mutations in the BRCA1 RING Domain. After cataloging the 14 missense mutations, I verified the credibility of these sources by looking for more information on the effects of these mutations using the Clinvar database. To obtain accurate diagrams of various protein structures, I used the Protein Data Bank.

Results

M18K mutation is seen on exon 2, with a 172T>A substitution, resulting in missense mutation in BRCA1 protein. This mutation was indicated as M18K. The location of M18K mutation was found to be 6 amino acids upstream to the conserved C3HC4 RING zinc finger domain of BRCA1. This missense mutation resulted in the replacement of the hydrophobic amino acid methionine (M) by the polar amino acid lysine (K). This change in the amino acid residue affected the tertiary protein folding of the RING domain of BRCA1, thus affecting its activity (Machackova et al., 2001). Mutation C64G causes the RING region to behave abnormally. In a normal situation the BRCA1 protein will be able to function properly because it is not deformed. However, this mutation caused a conserved cysteine to change to glycine at the 64th amino acid in the RING region of the BRCA1 protein. By switching one amino acid, a new 5' splice site is created in exon 5 and disrupted the old splice site which causes a 22-nucleotide deletion from the BRCA1 protein and codes for a deformed protein with 63 amino acids instead of the normal 1863 amino acids (Y. Yang et al., 2003). This effectively stops the function of the BRCA1

protein, and without the function of the BRCA1 protein, can cause hereditary breast cancer. The M18T mutation causes an increase in intrinsic protein ligase activity, which showed that it did not impair E3 ligase activity. However, this mutation did not increase IR resistance, meaning that it made the BRCA1 gene more likely to stay in the mutated form because the gene repair mechanism was affected by the mutation M18T (Ruffner et al., 2001). IR resistance is important because radiation introduces mutations in the genes, and without IR resistance, a repair mechanism will not repair the mutated gene. A decrease in IR resistance may cause breast cancer because there will be an accumulation of mutations in the gene due to the lack of radiation resistance. Mutation R71G does not cause E3 ligase ubiquitin to be disrupted, nor does it increase IR sensitivity (Ruffner et al., 2001). However, it does affect the donor splice site in exon 5 by mutating it. Additionally, 22 base pairs on Exon 5 were deleted introducing a stop codon to be created with the first few base pairs in Exon 6 at position 64 (Y. Yang et al., 2003). This causes the BRCA1 protein to be truncated and deformed so it will not be able to perform its functions. A deformed protein cannot do its function of gene repair and cell cycle regulation that leads to breast cancer. Mutation C44F causes the RING region to behave abnormally. In a normal situation the BARD1/BRCA1 complex will be able to function normally. However, this mutation causes the BRCA1 protein to not bind with BARD1 causing no E3 ligase activity. This is because this mutation mutates the C3HC4 RING finger canonical residue that is essential for binding zinc ions to keep the RING Finger structure together (Abkevich et al., 2004). Zinc binding residues are used for BRCA1 and BARD1 to attach to each other. If the BRCA1 does not have its Zinc ions properly placed, it will not be able to bind BARD1. This is a problem because there will not be any ubiquitination of target proteins which means no regulation of the cell cycle and can cause breast cancer.

In a normal situation BRCA1 and BARD1 will form a heterodimer that will degrade different proteins for cell cycle regulation, transcriptional regulation, and gene repair. However, C24R mutation causes a mutation on the fourth putative zinc binding residue which is detrimental to ubiquitination (Abkevich et al., 2004). This is because zinc binding is necessary for BRCA1 and BARD1 to form a heterodimer; without this zinc binding, ubiquitination of proteins will not occur. This will cause breast cancer because protein function and gene regulation will not happen as expected. Mutation C61G causes the RING region to be affected. In a normal situation, ubiquitination of different proteins helps regulate cellular processes such as protein degradation. However, this mutation causes E3 ubiquitin ligase activity in the BRCA1 gene to be stopped. This causes target proteins such as cell regulators to not be degraded and the BRCA1 protein itself is more prone to degradation. Additionally, this mutation disrupts the zinc binding site, causing the inactivation of E3 ligase activity. This mutation impairs the second G2 + M checkpoint of the cell cycle in the BRCA1 gene, causing cells to more likely be produced ectopic cells (Ruffner et al., 2001). This causes a more likely chance for breast cancer as E3 ligase activity is stopped. Mutation T37R causes the RING region to not function normally. In a normal situation BARD1 and BRCA1 can form a heterodimer and function as ubiquitin that destroys proteins to keep up with cell regulation. While the mutation does not affect the heterodimer formation of BRCA1 and BARD1, it impairs E2 binding which stops E3 ligase activity (Ruffner et al., 2001). Without ubiquitination, there will be no gene regulation leading to breast cancer.

Mutations C64Y, C39Y, C47G, C64R and C39S cause the RING region to behave abnormally. In a normal situation BRCA1 and BARD1 will form a heterodimer that will degrade proteins for cell

cycle regulation, transcription regulation on gene repair. However, this mutation causes all E3 ubiquitin ligase activity in the BRCA1 gene to be stopped (Ruffner et al., 2001). These mutations mutate Cysteine in the C3HC4 RING finger canonical residue that is essential for being zinc ions to keep the RING finger structure together (Abkevich et al., 2004). Zinc binding residues are used for BRCA1 and BARD1 to attach to each other. If the BRCA1 does not have its Zinc ions properly structured, it will not be able to bind BARD1. Zinc binding residues are used for BRCA1 and BARD1 to attach to each other. If the BRCA1 does not have its Zinc ions properly structured, it will not be able to bind BARD1. This causes breast cancer because the BRCA1/BARD1 complex will have been nulled.

The H41R mutation mutates the C3HC4 RING Finger canonical residue that is essential for being zinc ions to keep the RING finger structure together. This is caused because histidine is replaced by arginine at amino acid 41 (Abkevich et al., 2004). Zinc binding residues are used for BRCA1 and BARD1 to attach to each other. If the BRCA1 does not have its Zinc ions properly structured, it will not be able to bind BARD1. Zinc binding residues are used for BRCA1 and BARD1 to attach to each other. If the BRCA1 does not have its Zinc ions properly structured, it will not be able to bind BARD1.

Discussion

Missense Mutations in the RING Domain led to an amino acid change in the formation of the BRCA1 protein that caused a change in the protein structure. Zinc molecules cannot be held because of the mutated RING Finger and subsequently does not allow a heterodimer to form. With a heterodimer, there will also not be any E3 Ligase activity and keeps the gene unregulated. These mutations could be addressed through Gene therapy, the delivery of a nucleic acid-based drug to correct or destroy mutated cells. Recent studies have shown that inserting p53 gene into BCSC, the source of tumors, the spread of cancerous cells would stop (Zhang et al., 2025). This innovative approach can be a future treatment option for breast cancer.

References

1. Abkevich, V., Zharkikh, A., Deffenbaugh, A. M., Frank, D., Chen, Y., Shattuck, D., Skolnick, M. H., Gutin, A., & Tavtigian, S. V. (2004). Analysis of missense variation in human BRCA1 in the context of interspecific sequence variation. *Journal of Medical Genetics*, *41*(7), 492–507. https://doi.org/10.1136/jmg.2003.015867
2. Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *74*(3), 229–263. https://doi.org/https://doi.org/10.3322/caac.21834
3. Brzovic, P. S. ;, Rajagopal, P. ;, Hoyt, D. W. ;, King, M.-C. ;, & Klevit, R. E. (2001). *Full wwPDB NMR Structure Validation Report i ○ Title : Solution structure of the BRCA1/BARD1 RING-domain heterodimer*. https://doi.org/10.1038/nsb1001-833
4. Gorodetska, I., Kozeretska, I., & Dubrovska, A. (2019). BRCA genes: The role in genome stability, cancer stemness and therapy resistance. In *Journal of Cancer* (Vol. 10, Issue 9, pp. 2109–2127). Ivyspring International Publisher. https://doi.org/10.7150/jca.30410

5. Gracia, B., Montes, P., Gutierrez, A. M., Arun, B., & Karras, G. I. (2024). Protein-folding chaperones predict structure-function relationships and cancer risk in BRCA1 mutation carriers. *Cell Reports*, *43*(2). https://doi.org/10.1016/j.celrep.2024.113803
6. Iii, F., Le Trong, D. ;, Stenkamp, I. ;, & Klevit, R. E. ; (2023). *Full wwPDB X-ray Structure Validation Report i ○ Title : Crystal Structure of the BRCA1 Associated Ring Domain (BARD1) Tandem BRCT Domains*. https://doi.org/10.2210/pdb3FA2/pdb
7. Machackova, E., Damborsky, J., Valik, D., & Foretova, L. (2001). Novel germline BRCA1 and BRCA2 mutations in breast and breast/ovarian cancer families from the Czech Republic. *Human Mutation*, *18*(6), 545. https://doi.org/10.1002/HUMU.1232
8. Morris, J. R., Pangon, L., Boutell, C., Katagiri, T., Keep, N. H., & Solomon, E. (2006). Genetic analysis of BRCA1 ubiquitin ligase activity and its relationship to breast cancer susceptibility. *Human Molecular Genetics*, *15*(4), 599–606. https://doi.org/10.1093/hmg/ddi476
9. Ruffner, H., Joazeiro, C. A. P., Hemmati, D., Hunter, T., & Verma, I. M. (2001). *Cancer-predisposing mutations within the RING domain of BRCA1: Loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity*. https://doi.org/10.1073/pnas.081068398
10. Yang, Q., Zhao, J., Chen, D., & Wang, Y. (2021). E3 ubiquitin ligases: styles, structures and functions. In *Molecular Biomedicine* (Vol. 2, Issue 1). Springer. https://doi.org/10.1186/s43556-021-00043-2
11. Yang, Y., Swaminathan, S., Martin, B. K., & Sharan, S. K. (2003). Aberrant splicing induced by missense mutations in BRCA1: clues from a humanized mouse model. *Human Molecular Genetics*, *12*(17), 2121–2131. https://doi.org/10.1093/hmg/ddg222
12. Zhang, Z., Li, T., Li, Y., Wang, X., Liu, H., Shen, X., Xu, A., Xia, T., & Xu, B. (2025). Cancer stem cell specificity as new targets in breast tumor treatment. In *Oncology Research* (Vol. 33, Issue 4, pp. 811–819). Tech Science Press. https://doi.org/10.32604/or.2024.050505