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International Journal of Chronic Diseases & Therapy (IJCDT)
ISSN: 2572-7613

Mechanism Based Precision Diagnostics for BRCA1-Associated TNBC

Research Article

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Abstract

BRCA1 gene mutations result in an increased risk for TNBC, which has a high mortality rate, and currently, there are no targeted therapies nor biomarkers for early detection. Various BRCA1 mutations have been found throughout the BRCA1 coding region; some are germline pathogenic variants, and others are known as variants of uncertain significance (VUS), whose cancer risk is unknown. Our previous work suggested that BRCA1/1a proteins interact with a nuclear chaperone Ubc9 and function as a growth/tumor suppressor in TNBC cells, unlike the pathogenic variants. This work is based on the hypothesis that BRCA1 may contain driver mutations that may cause loss of function and TNBC development or passenger mutations that maintain WT BRCA1 function. We tested this hypothesis by transfecting BRCA1/1a and its various mutant (K109R, C61G, I26A) plasmids into patient derived BRCA1 mutant TNBC cells. We studied the various functions such as in vivo association of BRCA1 with Ubc9 by immunofluorescence analysis, induction of apoptosis using chromatin condensation, growth/tumor suppression activity using colony suppression and scratch migration assay. Our results demonstrate for the first time BRCA1/1a I26A variant to function like WT BRCA1/1a, unlike K109R and C61G mutants. The I26A mutant (which lacks E3 Ubiquitin ligase activity possesses homologous recombination (HR) activity and induces SIRT1 expression) was found to bind Ubc9, inhibit growth/migration of TNBC cells and trigger apoptosis. This is the first study demonstrating the physiological link between Ubc9 binding, HR activity, loss of BARD1- dependent E3 Ubiquitin ligase activity, growth/tumor suppression, apoptosis, and SIRT1 induction by I26A mutant BRCA1/1a protein in TNBC cells. Clinically, the ability to predict which of these mutations can result in TNBC offers unprecedented prospects for early detection and cancer prevention. This study in the future will accelerate precision oncology, provide guidelines for early detection, prevention, and reduction in cancer health disparities.

Keywords: BRCA1; BRCA1a; Ubc9; TNBC; BRCA1 Variants; RING Domain; VUS; Apoptosis; Precision Diagnosis.

Introduction

Breast cancer is the second leading cause of cancer-related deaths in the United States, with 42,170 women expected to die from breast cancer in 2020 [1]. There are two main subtypes of breast cancer in situ, ductal carcinoma, and lobular carcinoma. Though these subtypes are benign, they may be associated with an increased risk of invasive breast cancers. Roughly 81% of breast cancers are invasive and are comprised of 4 main molecular subtypes, Luminal A(HR+/HER2-), Luminal B (HR+/HER2+), Basal-Like (HR-/HER2-), and HER2-enriched as well as over 21 histological subtypes [2, 3]. Due to breast cancer's variability and subtypes, the average 5-year survival rate is 90% [4]. However, if distant metastasis occurs, the five-year survival rate drastically declines to 28.1% [4]. Triple-Negative Breast Cancer (TNBC) is of the Basal-like subtype of breast cancers [2]. TNBC is a heterogeneous disease based on distinct gene expression profiles and makes up 10-20% of all breast cancers [5]. TNBC is a phenotypically aggressive cancer with early-onset, higher mean tumor size, tumor grade, node positivity, recurrence, distant metastasis, and

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Received: March 08, 2021
Accepted: March 27, 2021
Published: April 02, 2021

Citation: Jingyao Xu, Alexis Clark, Yunlong Qin, Kristiana McLarty, Sothivin Lanh, Veena N Rao. Mechanism Based Precision Diagnostics for BRCA1-Associated TNBC. *Int J Chronic Dis Ther.* 2021;7(1):111-117. **doi:** http://dx.doi.org/10.19070/2572-7613-2000023

poorer survival compared to other breast cancers [6]. TNBCs lack estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) [6]. Due to the absence of hormone receptors in TNBC, there are currently no targeted therapies available [6]. Lehmann classification of TNBC includes six molecular subtypes: basal-like (BL1 and BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) subtypes. BRCA1 mutant TNBCs usually fall in the BL1 and BL2 subtypes. The Basal-like subtype has a high reoccurrence rate and distant metastasis mainly to the brain and lung [5-9]. This contributes to the poor prognosis and higher mortality in basal-like tumors. African American women (AA) have the highest rate of metastatic TNBC and the Basal-like subtype compared to other ethnic groups [5]. TNBCs of BL1 and BL2 subtypes with a BRCA1 mutation are typically sensitive to platinum-based chemotherapy such as cisplatin [8]. Although there have been improved TNBC outcomes, distant recurrence due to metastasis is common and incurable [9]. Therefore, there is an urgency to identify pertinent biomarkers to develop targeted therapy for TNBC. BRCA1 gene was cloned by Miki et al. in 1994 [10]. In TNBC, the BRCA1 gene is mutated, expressed at low levels, or localized abnormally [6, 11]. We have cloned two major conserved BRCA1 splice variants BRCA1a/p110 and BRCA1b/p100 [12, 13], expressed at lower levels in breast tumors [14-17]. Furthermore, we have found BRCA1a protein to induce apoptosis and suppress in vivo tumor growth of TNBC, ovarian, and prostate cancer cells [18, 19].The BRCA1 protein is structured into highly conserved functional domains located at the ends of the protein, an N- terminal RING domain, and two C- terminal BRCT repeats that participate in transcriptional activation [12, 17, 20, 21]. *In vivo*, the BRCA1 RING domain forms a heterodimeric complex with BARD1 that performs E3 Ubiquitin ligase activity [22]. BRCA1/ BRCA1a and BRCA1b are nuclear-cytoplasmic shuttling proteins that also localize to the mitochondria [12, 17, 20, 21]. Nuclear export signal (NES) within the RING domain interacts with BARD to facilitate nuclear transport of BRCA1 proteins [23]. The BARD-BRCA1 heterodimer is an E3 Ubiquitin ligase, and ER-α is a substrate *in vitro* [23-25]. Most BRCA1 mutations are frameshifts or nonsense mutations that lead to premature termination, truncated protein length, and impaired function [21]. Normally, BRCA1 proteins interact with the sole SUMO E2-conjugating enzyme Ubc9 [26]. This facilitates both the entry of BRCA1 proteins to the nucleus and mediates activation of ER-α [29]. The disease-associated mutants (C61G and K109R) do not bind Ubc9, are mislocalized in the cytoplasm, and have lost their growth /tumor suppressor function [27], which may result in TNBC. For the first time, a novel molecular mechanism for the development of TNBC due to BRCA1 mutation has been identified [26]. BRCA1 mutations that result in chain termination and a truncated protein are associated with an increased risk of breast and ovarian cancer [10, 21, 28]. Current testing for BRCA1 is based on DNA testing. This can result in positive, negative, and variants of uncertain significance (VUS). VUS remain a challenge as they are difficult to classify as benign or pathogenic. Several functional assays have been developed to study the wild-type BRCA1/1a protein functions [26]. I26A is a missense mutation in the BRCA1 RING domain. Studies have shown that the I26A mutation lacks E3 Ubiquitin ligase activity, does not ubiquitinate ER-α but repairs chromosome breaks by homologous recombination similar to wild-type BRCA1 [22]. ER-α is a putative substrate for BRCA1 Ubiquitin ligase [25]. SUMOylation has been shown to have similar protective effects against proteasomal degradation in pathological disease manifestations [29]. The SUMOylation and deSUMOylation process contribute to the stability and subcellular localization of factors in chronic diseases [29]. The SUMO E2 -conjugating enzyme Ubc9 catalyzes SUMO conjugation. We have shown that the RING finger domain at the N-terminal domain of BRCA1 binds Ubc9 to activate ER-α. BRCA1 disease-associated mutants do not bind to Ubc9 resulting in cytoplasmic localization of BRCA1 proteins [27]. Furthermore, BRCA1 was shown to possess a SUMO-1 and Ubc9 – dependent E3 Ubiquitin ligase activity on ERα [26]. These studies indicate that BRCA1 is a putative SUMO-1 and Ubc9-dependent E3 Ubiquitin ligase for ER-α [26]. BRCA1/1a with K109R and C61G mutations in the RING domain does not bind Ubc9 or regulate ER-α activity in breast cancer cells [26, 27]. These mutant proteins display deregulated Caveolin and VEGF expression in TNBC and HGSOC [38]. This implicates that the K109R and C61G mutations have lost WT BRCA1 function, resulting in TNBC and ovarian cancers. Ubc9 activity is involved in breast cancer cell migration, tumor progression, and resistance to chemotherapy [30-37]. Among the Nigerian black women population with breast cancer, Ubc9 expression was linked to poor clinical outcome and survival [37]. Our recent studies show that knockdown of Ubc9 inhibits proliferation and migration of BRCA1 mutant TNBC and HGSOC cells, suggesting a critical role in epithelial to mesenchymal transition (EMT) in TNBC and HGSOC [30]. EMT is described as a process where epithelial cells migrate to distal sites and thereby cause cancer metastasis. Sirtuins (SIRT) are NAD+ dependent proteins found in humans and mammals that function as cellular homeostatic regulators linked to genomic stability and ER-α repression [39-41]. SIRT1 possesses histone deacetylase activity, and dysregulation can be a biomarker for pathogenicity in TNBC [42]. Functional BRCA1 binds to the promoter of SIRT1, inhibiting the expression of Survivin by modification of histone H3 [43-45]. SIRT1 plays a role in subtype-specific targeted acetylation in breast cancer [46]. Histone acetylation is an important process by which lysine residues in the N-terminal are acetylated, resulting in gene modulation. However, in many human breast cancers such as TNBC, there are lower levels of SIRT1 and increased Survivin levels promoting tumorigenesis [39, 40, 47]. Our group has previously reported that the BRCA1 RING domain, unlike K109 R and cancer-predisposing mutant C61G proteins, interacts with the sole SUMO E2-conjugating enzyme Ubc9 [27]. This facilitates both the entry of BRCA1 proteins to the nucleus and mediates activation of ER-α. The disease-associated mutants do not bind Ubc9 and are mislocalized in the cytoplasm [26, 27, 48]. Therefore, they have lost their growth/tumor suppressor function. We have identified a new nuclear trafficking pathway, and malfunction of this by BRCA1 dysfunction can result in TNBC. Our previous results showed BRCA1/1a and its I26A mutant proteins but not the pathogenic BRCA1/1a mutants which do not bind Ubc9 to induce SIRT1 expression in TNBC cells [7]. Knockdown of Ubc9 in these TNBC cells resulted in expression of SIRT1 suggesting a role for Ubc9 in promoting distant metastasis [7]. Several mutations in the BRCA1 RING domain have been identified; however, their role in TNBC has yet to be elucidated. This work is based on the hypothesis that BRCA1 is a tumor suppressor gene, and its RING domain can harbor several mutations, some of which are driver mutations that have lost BRCA1 function resulting in TNBC and others function like WT BRCA1. We plan to test this hypothesis by introducing C61G, K109R, and I26A mutations into TNBC cells and study the various functions like association

with Ubc9, induction of apoptosis, inhibition of cell proliferation and migration. This study will demonstrate whether BRCA1/1a I26A proteins function like WT BRCA1 or not. Clinically, the ability to predict which of these mutant BRCA1 proteins can result in TNBC offers unprecedented prospects for early detection and cancer prevention. This study will accelerate precision medicine and reduce cancer health disparities in health outcomes.

Materials And Methods

Expression constructs. Full-length BRCA1a, BRCA1a Mut #1, BRCA1a Mut #4, BRCA1a Mut #8, BRCA1a Mut # 9, BRCA1, BRCA1 Mut #1, BRCA1 Mut #4, BRCA1 Mut#9 or pcDNA3 vectors as described previously [26]. Point mutations were generated as described previously [26].

Cell culture. HCC1937, MCF7, and Cos-1 cells were obtained from American Type Culture Collection (Rockville, MD, USA) and cultivated as described previously [13, 26].

Antibodies and Reagents. The antibodies used in this study were MS110 ascites (Ab1, EMD Chemicals), Ubc9 (N-15, Sc5231 polyclonal antibodies, Santa Cruz Biotechnology).

Immunofluorescence microscopy and live imaging. To analyze the subcellular localization, HCC1937 and Cos-1 cells were seeded into 6-well plates a day before transfection with BRCA1, BRCA1a, and their respective mutant plasmids. The DNA was stained with Hoechst dye and, after 24 hrs., the cells were visualized under a fluorescent microscope (Olympus, 20X lens) as described previously [26, 27].

Growth suppression studies. HCC1937 and MCF7 cells were plated at a concentration of 1.5X105 cells per 100mm plate in 10ml of complete media and transfected with either pcDNA3 or pcDNA3 BRCA1/1a, pcDNA3 BRCA1/1a I26A, pcDNA3 BRCA1a Mut#1, and pcDNA3 BRCA1a Mut#4 by using the X-tremeGENE9 from Roche. 42 hrs. later, cells were trypsinized, and after 24 hrs., the medium was replaced by one containing 200µg/ml G418. Cells were fed fresh medium containing G418 every 3-4 days. Cells were stained for colonies approximately 20 days after transfections using crystal violet blue as described previously [13].

Soft agar assay. Soft agar assay was carried out using 0.3% agar/DMEM/10% FBS and plated on a base of 0.5% agar/DMEM as described previously [13, 18]. MCF7 and HCC1937 cells were plated at a concentration of 1X104 per 35mm plate in soft agar containing complete media and kept at 37°C in a CO_2 incubator. A few drops of media were added every week. Colonies more than 80µm in diameter were scored after three weeks. Each soft agar assay was performed in triplicate.

Scratch migration assay. To perform scratch assays, 2X10 5 HCC1937 stable cells that were transfected with pcDNA3, BRCA1 and BRCA1 Mut#1, BRCA1 Mut#9, BRCA1a, and BRCA1a Mut#9 using the Qiagen kit as described previously (30) were plated into a 6-well cell culture plates. After 24 hrs., a 1mm wide scratch was made across the cell monolayer using a sterile 200µl pipette tip. Cells were grown in normal medium for 24 hrs. to 48 hrs. Plates were photographed at 0 hr. immediately after scratch and 24 hrs. or 48 hrs., following scratch as needed. All experiments were repeated at least twice [26].

Chromatin condensation. HCC1937 and MCF7 cells were seeded on glass coverslips in 6-well plates a day before transfection. The following day, pcDNA3, BRCA1a, Mut#1, Mut#4, Mut#8 (K119R), Mut#9, and BRCA1, Mut#1, and Mut#4 were transfected into the cells using Lipofectamine 2000 transfection reagent (Invitrogen). 24 hrs. later, cells were fixed in methanol: glacial acetic acid (3:1) at –18oC for 30 min. The cells were washed in PBS and stained with 8µg/ml Hoechst 33258 (Pentahydrate, Life technologies) for 1 hour in the dark. The coverslips were rinsed in water and mounted with fluorescence mounting media. The images were taken using a fluorescent microscope (100X, oil Olympus) as described previously [18].

Results And Discussion

BRCA1a I26A (Mut#9) associates with Ubc9 like Wild type BRCA1/1a proteins

BRCA1/1a proteins have been found to interact in vivo and in vitro with SUMO conjugating enzyme Ubc9, which facilitates entry of these proteins to the nucleus [26, 27]. BRCA1/1a Mut#1 (K109R) and pathogenic Mut #4 (C61G) were unable to bind to Ubc9 and were stalled in the cytoplasm [26, 27]. To study whether BRCA1a I26A missense mutation associates with Ubc9 in vivo, we transfected BRCA1a, BRCA1a Mut#1, BRCA1a Mut#4, and BRCA1a Mut#9 into Cos-1 cells and visualized the colocalization using immunofluorescence analysis. Our results suggested in vivo association of BRCA1a and BRCA1a Mut #9, unlike BRCA1a Mut#1 and BRCA1a Mut #4 with Ubc9 (Figure 1). These results support our hypothesis that BRCA1a Mut #9 associates with Ubc9 to the same degree as Wild-type BRCA1/1a protein.

BRCA1a I26A (Mut#9) induces apoptosis like Wild type BRCA1/BRCA1a proteins in TNBC cells

BRCA1/1a proteins have previously been shown to induce apoptosis in human breast cancer cells [18]. To examine whether BRCA1/1a Mut #1, Mut#4, Mut #8, and Mut #9 induce apoptosis in MCF-7/HCC1937 breast cancer cells, we subjected the cells to phase-contrast microscopy after staining the cells with Hoechst 33258 as described previously [18]. The majority of the nuclei of BRCA1/1a and BRCA1a Mut# 9 showed strong chromatin condensation and nuclear degradation into small spherical nuclear particles of condensed chromatin characteristic of apoptosis (Figure 2A, B), whereas the BRCA1/1a Mut#1, BRCA1/1a Mut#4, and BRCA1a Mut # 8 did not show any significant changes with the staining pattern. These results indicate that BRCA1a Mut#9 induces cell death in TNBC cells like WT BRCA1/1a protein.

BRCA1/1a I26A (Mut#9) suppresses growth of MCF7 and TNBC cells like Wild type BRCA1/1a proteins

BRCA1/1a proteins are known to function as growth suppressors in TNBC, ovarian, and prostate cancer cells [19]. In an attempt to understand whether BRCA1/1a Mut#9 functions as a growth suppressor in MCF7 and TNBC cells similar to BRCA1/1a, we transfected BRCA1/1a, BRCA1/1a Mut #9, BRCA1a Mut#1, BRCA1a Mut# 4 plasmids into MCF7 and BRCA1 mutant TNBC

Figure 1. Colocalization of BRCA1a and BRCA1a Mut#9 unlike BRCA1a Mut#1 and BRCA1a Mut#4 with Ubc9 in Cos-1 cells as detected by immunofluorescence analysis. Cos-1 cells were seeded in six-well plates. The nuclei were visualized using DNA staining dye DAPI. Cells were fixed in methanol and probed with Ubc9 (Santa Cruz, 1/250) and BRCA1 (1/250) followed by Alexa Fluor 568/488 labeled secondary antibody (Invitrogen, 1/200) staining as described (26). The images were taken using fluorescent microscope (100X, oil Olympus).

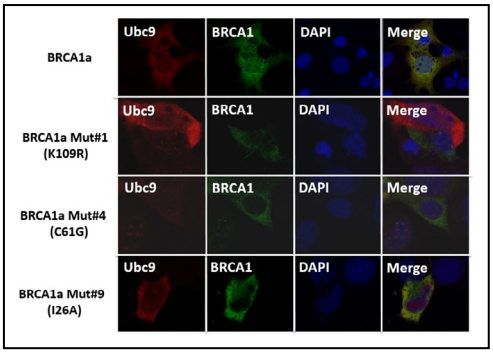


Figure 2A. BRCA1 unlike BRCA1 Mut #1 and BRCA1 Mut #4 induce apoptosis in MCF7 breast cancer cells, as shown by chromatin condensation. MCF7 stable cell lines, pcDNA3, pcDNA3 BRCA1, pcDNA3 BRCA1 Mut#1 and pcDNA3 BRCA1 Mut#4 were seeded into 6-well plates at a density of 1x 105/well. The nuclei were visualized with DNA staining dye Hoechst (Invitrogen). The nuclei images were taken using a fluorescent microscope (20X) (Olympus) as described (26).

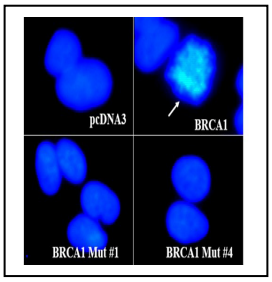


Figure 2B. BRCA1a Mut#9 induces apoptosis like BRCA1a in TNBC cells. HCC1937 cells were seeded into the 6-well plates the day before transfection. The following day, pcDNA3, BRCA1a, Mut#1, Mut#4, Mut#8 and Mut#9 were transfected into the cells using Lipofectamine 2000 transfection reagent (Invitrogen). The twenty-four hours later, the transfectants were stained with Hoechst 33258 (Pentahydrate, Life technologies) for an hour. The images were taken using fluorescent microscope (100X, oil Olympus). Experiment was done as described previously (18).

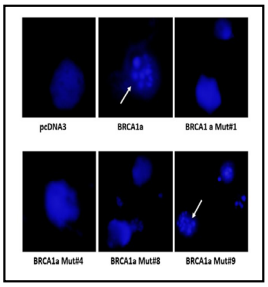
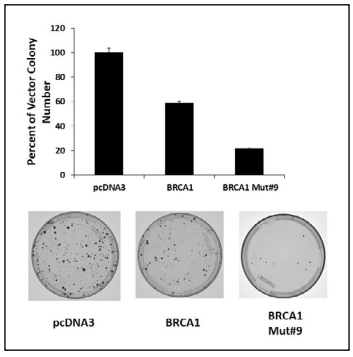


Figure 3A. BRCA1 Mut#9 (I26A) suppresses growth of MCF7 cells similar to WT BRCA1. MCF7 cells were transfected with pcDNA3 or pcDNA3-BRCA1 or pcDNA3-BRCA1 I26A and selected with G418 and the G418 resistant colonies were stained with crystal violet and counted as described (13). The experiment was repeated three times and the bars shown represent s.d.



cell line HCC1937 and subjected them to colony suppression assay as described previously [13]. Overexpression of BRCA1 Mut#9 resulted in a reduction in the number of G418 resistant colonies like BRCA1 in MCF7 cells (Figure 3A). Overexpression of BRCA1a Mut# 9 in HCC1937 cells once again resulted in a reduction in the number of G418 resistant colonies like Wild type BRCA1 a (Fig. 3B). These results once again suggest BRCA1/1a Mut #9 to function as a growth suppressor of MCF7 and TNBC cells like BRCA1/1a proteins.

BRCA1/1a I26A (Mut#9) inhibits migration of TNBC cells like Wild type BRCA1/1a proteins

BRCA1/1a proteins have been shown to function as growth/tumor suppressors of breast, ovarian, and prostate cancer cells [19, 27, 30]. Both BRCA1/1a K109R and disease-associated C61G mutants fail to function as growth / tumor suppressors of TNBC and ovarian cancer cells [19, 28]. To study the role of BRCA1/1a I26A mutant in TNBC cell migration, we subjected the HCC1937 BRCA1/1a stable cell lines to scratch migration assay as described previously by us [30]. As expected, BRCA1/1a I26A mutant inhibited the migration of HCC1937 cells like WT BRCA1/1a (Figure 4A and B). These results once again reinforce the functional similarity of BRCA1/1a I26A to Wild type BRCA1/1a proteins.

Conclusions

Deleterious mutations in the BRCA1 gene confer a significantly increased risk for TNBC and those involving the N- or C-terminal regions are associated with aggressive tumors. The identifica-

Figure 3B. BRCA1a Mut#9 (I26A) suppresses the growth of HCC1937 TNBC cells like BRCA1a unlike BRCA1 Mut#1 (K109R) and BRCA1a Mut#4 (C61G). The transient transfection of HCC1937 cells were carried out using X- treme GENE 9-mediated transfection using pcDNA3, BRCA1a, BRCA1a Mut#9, BRCA1a Mut#1 and BRCA1a Mut#4. The cells were selected using G418 and the number of colonies were counted after staining with crystal violet as described (13). The number of colonies obtained by vector control was considered as 100%. The experiment was repeated three times and the bars shown represent s.d.

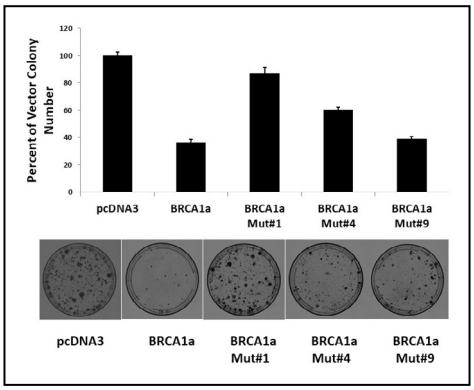


Figure 4A. BRCA1 Mut#9 I26A mutant suppresses migration of HCC1937 cells like WT BRCA1 using scratch migration assay. HCC1937 stable cells transfected with pcDNA3 or pcDNA3-BRCA1 or pcDNA3-BRCA1 Mut#1 or pcDNA3-BRCA1 Mut#9 were seeded into 6-well cell culture plates and 1mm wide scratch was made across the cell layer using a sterile tip. The cells were grown for 24 hrs. and photographed at 0hr and 24 hrs. after scratch as described previously (26).

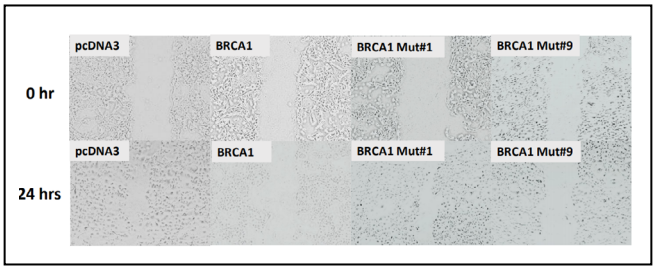
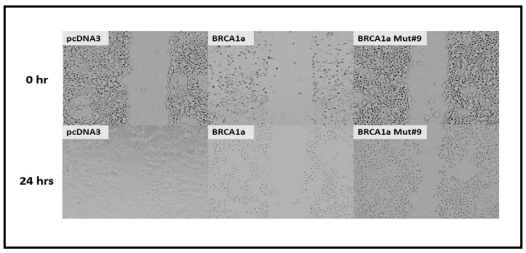


Figure 4B. BRCA1a Mut#9 (I26A) suppresses migration of HCC1937 cells like WT BRCA1a using scratch migration assay. HCC1937 stable cells transfected with pcDNA3 or pcDNA3-BRCA1a or pcDNA3-BRCA1a Mut#9 were seeded into 6-well cell culture plates and 1mm wide scratch was made across the cell layer using a sterile tip. The cells were grown for 24 hrs. and photographed at 0 hr and 24 hrs. after scratch as described previously (26).



tion of a disease-associated mutation has important implications for the clinical management of patients in guiding strategies for prevention and early detection of TNBC. BRCA1 testing can result in three different scenarios: 1) Negative, no mutation found; 2) Positive, a pathogenic mutation that is disease-associated; 3) VUS, BRCA1 sequence variants of unknown clinical significance. It is not clear if VUS mutation is benign or can cause cancer. VUS occur disproportionately in AA women, which can complicate genetic counseling, management, and clinical care for these patients [51]. Testing frequently detects VUS, which is a challenge to clinicians when determining how to appropriately guide the patient due to inconclusive test results leading to issues in risk assessment, counseling, and preventive care. The majority of the BRCA1 pathogenic mutations that have been studied are defective in homologous recombination DNA repair, and recently BRCA1 VUS were functionally categorized based on this aspect [52]. We have developed functional assays (BRCA1 function-based cellular assays patent number 8,372,580) to study mutant BRCA1 protein function on TNBC development. These assays can provide a framework for genome-wide pathogenicity assignment. They can be used to screen BRCA1 mimetic drugs for targeted therapy of TNBC, a disease that currently has no targeted treatments available. It will also lead to the discovery of personalized, targeted therapy for TNBC, thus reducing and ultimately eliminating the mortality associated with these aggressive breast cancers in young AA women.

Our data suggests for the first time that BRCA1/1a I26A mutant to be a passenger mutant, unlike disease-associated C61G and K109R mutants using patient-derived BRCA1 mutant TNBC cells. These results can be used in the future to classify VUS mutations to its clinical significance and stratify risk for TNBC development in patients who carry these mutations. It also suggests for the first time that Ubc9 binding, growth/tumor suppression, induction of apoptosis, and SIRT1 induction [7] of I26A mutant BRCA1 protein in TNBC cells. Since I26A mutant possesses HR activity and lacks BARD1- dependent E3 ubiquitin ligase activity [53], it suggests BRCA1 proteins to function as tumor suppressors in TNBC via tethering Ubc9. This study also highlights the patients' need for genetic counseling when seeking treatment options to distinguish between loss of function BRCA1 mutations in addition to mutations that have no clinical significance. The results of this study will further advance precision oncology, providing guidelines for early detection, prevention, and reduction in cancer health disparities. These breakthrough findings provide an opportunity to make progress in precision medicine initiative in oncology which is focused on mechanism-based cancer diagnostics and therapies.

Acknowledgements

We thank all the members of Drs. Rao and Reddy labs for their help. We thank RCMI core facilities at Morehouse School of Medicine, for their assistance. This work was supported in part by Georgia Cancer Coalition Distinguished Cancer Scholar award, NIHMD research endowment award 2S21MD000101, U54 MD007602 and U54 CA118638 to V.N.R. V.N.R's lab was also supported in part by funds from the VOYA foundation and Breast cancer partnership grant It's the Journey Inc, a Cure in our lifetime and Georgia CORE.

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