READTHROUGH COMPOUNDS AS SUPPRESSION AGENTS OF NONSENSE MUTATIONS IN *BRCA1*

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INTRODUCTION AND OBJECTIVES

Mutations in *BRCA1* are responsible for most cases of hereditary breast and ovarian cancer syndrome (HBOC). Nonsense variants account for ~ 12% of mutations in *BRCA1* gene; they are characterized by the appearance of a premature stop codon (PTC), generating a truncated protein. Different studies have shown that some compounds, like aminoglycosides, can induce readthrough of premature stop codons. The suppression of the PTC potentially restores the function of the protein. Given the lack of alternatives to the treatment of HBOC, the use of these compounds may represent an important strategy for the prevention of hereditary breast and ovarian cancer, and also another forms of hereditary cancer associated with nonsense mutations. Our study intends to evaluate the use of readthrough compounds on the restoration of tumor suppressor activity of nonsense variants of the *BRCA1* gene.

MATERIAL AND METHODS

Twelve variants coding PTC in the *BRCA1* C-terminus were selected for the study. Variants were generated and cloned into pQCXIH, a retroviral vector, in a fusion with EGFP or with GAL4 DBD. HeLa cells constitutively expressing the nonsense variants were tested in the presence and absence of G418 (an aminoglycoside) and evaluated for full-length protein synthesis restoration using immunoblotting (IB), flow citometry (FC) and confocal microscopy (CM). Functional restoration was also assayed by investigating readthrough-BRCA1 *ability to interact with CtIP. BRCA1* missense variants representing the most probable acquired mutations consequence of the readthrough event of the original 12 nonsense mutants were identified. Nineteen variants were generated and cloned into pcDNA3 vector in a fusion with GAL4 DBD to evaluate their impact in *BRCA1* biological function. CRISPR/Cas9 technology was used to develop human cell lines carrying nonsense mutations in *BRCA1* genomic loci. Guide RNAs (SX1 and SX2) were designed and cloned into pX458 vector. Efficacy of cleavage was then evaluated in MCF-7 cells. Donor DNA carrying a nonsense mutation (S1457X) was co-transfected with the guide RNA SX1 in MCF-7, MDA-MB-231, T47D and HeLa cells. Cellular cloning was performed by limiting dilution.

RESULTS AND CONCLUSION

Human cells showed full-length protein levels partially restored after a 48h treatment with G418 when evaluated by FC and CM. Among tested variants, S1457X showed the best restoration profile. We were unable to detect the full-length protein by IB. However, restoration of full-length protein levels does not reflect their biological functional status. To address this problem, we used BRCA1 ability to interact with CtIP to evaluate this issue. Functional restoration was observed on S1457X, W1508X e Q1785X when treated with G418. To assess PTC readthrough in a more comprehensive approach, we pursued to develop *BRCA1* point-mutated cell lines using CRISPR/Cas9 technology. Our data suggest that the loss of *BRCA1* $^{\text{wild type}}$ could induce a premature senescent phenotype in MCF-7 cells. Using p53 deficient cell lines (MDA-MB-231, T47D and HeLa) we were able to isolate cells edited by Cas9, but not clones carrying the S1457X mutation in *BRCA1* gene.

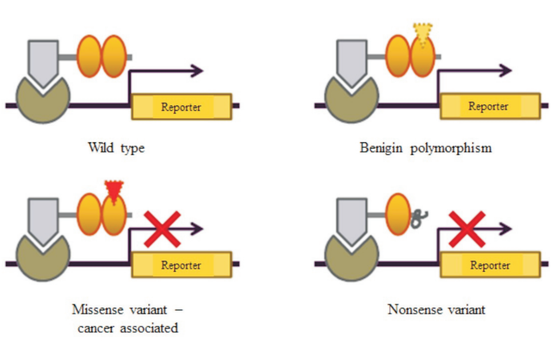

Figure 1: Schematic representation of transcriptional activation assay. (A) Represents the behavior of wild type protein; (B), a benign polymorphism; (C), a missense variant (cancer associated) and (D), a nonsense variant (cancer associated).

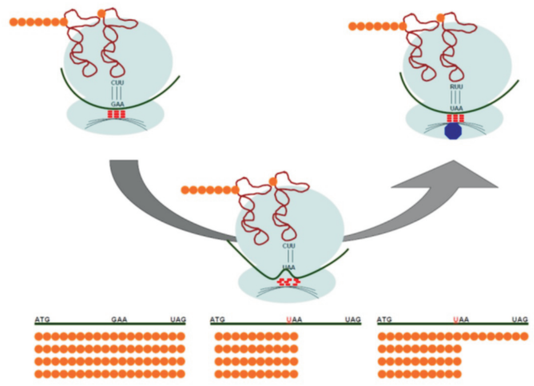

Figure 2: Schematic representation of aminoglycosides readthrough induction. The model illustrates the action of aminoglycosides on the ribosome during the translation process.

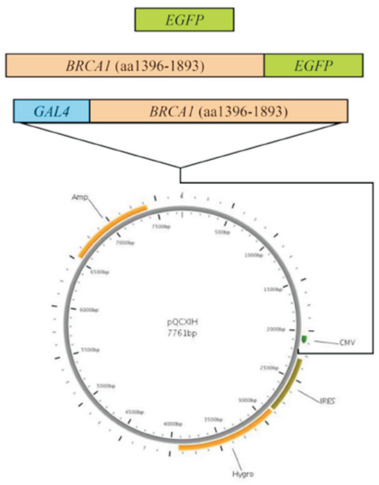

Figure 3: Clonning vector. The coding sequences for fusion proteins (EGFP in green, BRCA1 aa1396-1863 in orange and GAL4DBD in blue), including selected variants, were initially cloned using the TOPO ® cloning system. Subsequently, they were subcloned into pQCXIH.

Key words: *BRCA1*, nonsense, readthrough, CRISPR/Cas9.

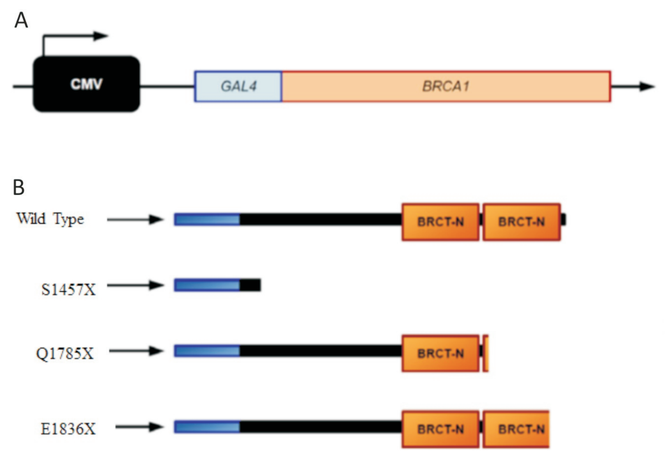

Figure 4: Schematic representation of GAL4:BRCA1 expression model. The promoter region (CMV) and *GAL4:BRCA1* DNA sequence were transducted in HeLa cells. Expression of these sequences result in different protein lengths according to the mutation position.

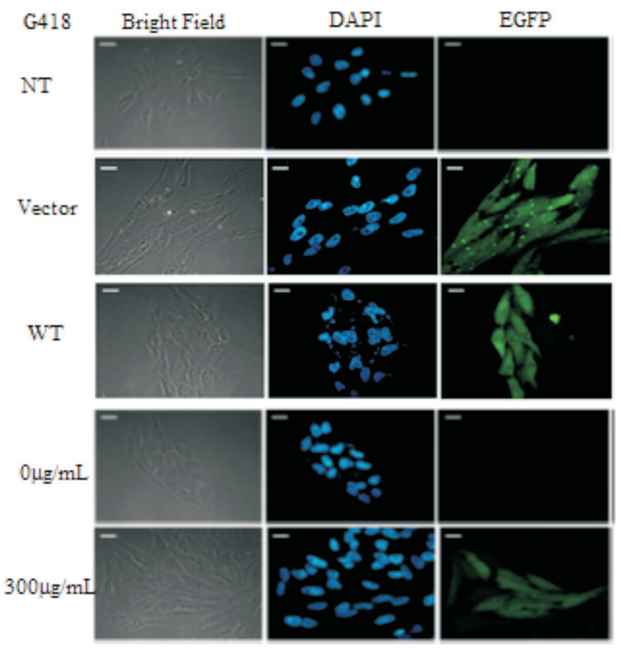

Figure 5: Readthrough analysis by confocal microscopy. The panel displays an experiment of induced readthrough (48 hours after treatment). Cells transiently transfected with wild type construct show a constitutive expression of BRCA1:EGFP. Cells transfected with *BRCA1* variant S1457X construct only reveals EGFP signal in the presence of 300µg/mL G418.

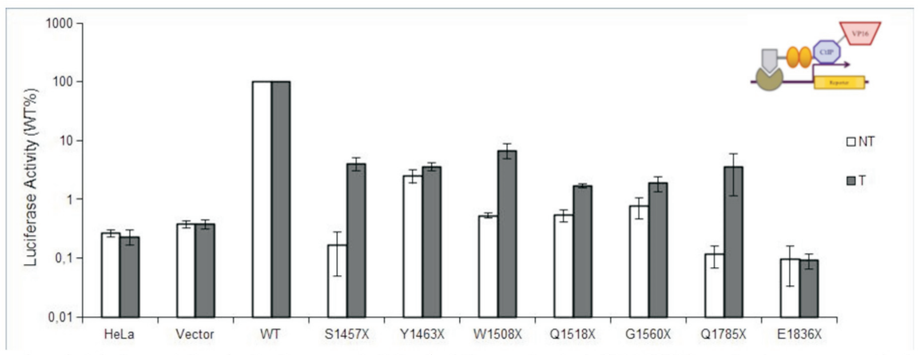

Figure 6: Readthrough analysis by transcriptional activation assay. Activity of wild type and variants GAL4:BRCA1 constructs was accessed by its interaction with CtIP fusioned with VP16. All variants showed very low activity without G418 and a increase in the activity after treatment (T).

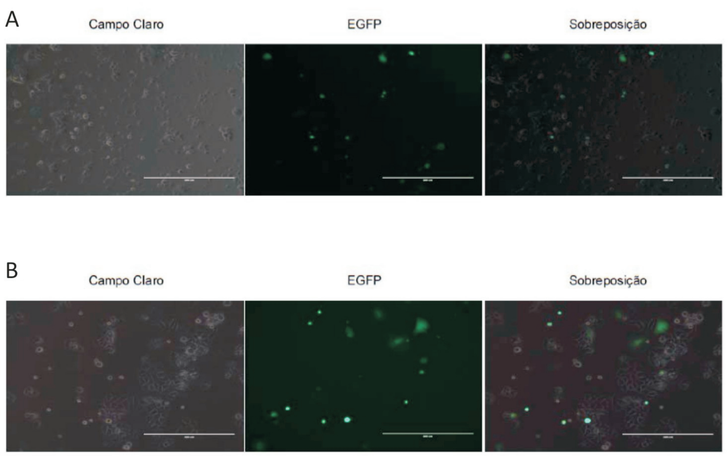

Figure 7: CRISPR/Cas9 genomic edition in MCF-7 cell line. The panel displays an experiment of genomic edition via CRISPR/Cas9 technology (24 hours (A) and 48 hours (B) after transfection). Cells transiently transfected with guide RNA SX1 construct and single strand donor DNA show a constitutive expression of EGFP.

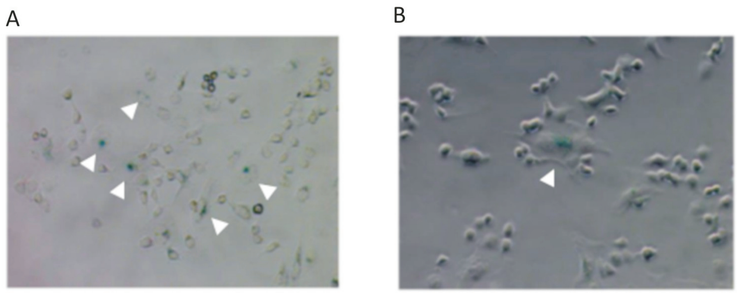

Figure 8: β-galactosidase assay. β-galactosidase activity was visualized in transiently cotransfected MCF-7 cells.

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