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

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INTRODUCTION AND OBJECTIVE

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

Four variants coding PTC in the BRCA1 C-terminus (S1457X, Y1463X, Q1785X and E1836X) 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 evaluated in the presence and absence of G418 by immunoblotting (IB), flow citometry (FC), confocal microscopy (CM) and were also functionally assayed investigating their ability to interact with CtIP. CRISPR/Cas9 technology was used to develop human cell lines carrying nonsense mutations in *BRCA1* genomic loci. Guide RNAs were designed and cloned into pX458 vector. Efficacy of cleavage was then evaluated in MCF-7 cells. Donor DNA carrying S1457X mutation was co-transfected with the guide RNA SX1 in MCF-7 cells and cloned 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 status. We were unable to detect the full-length protein by IB. However, restoration of full-length protein levels does not reflect the biological functional status. To address this problem, BRCA1 ability to interact with CtIP was used to evaluate this issue. Function restoration was observed on both S1457X e Q1785X when treated with G418. To assess PTC readthrough in a more comprehensive approach, we are developing *BRCA1* point-mutated cell lines using CRISPR/Cas9 technology. This leads to a model that resembles to the actual cellular status, enabling proper evaluation of BRCA1 tumor suppressor ability in response to DNA damage. Interestingly, primary data suggest that the loss of *BRCA1* $^{\text{wild type}}$ could induce a premature senescent phenotype in MCF-7 cells.

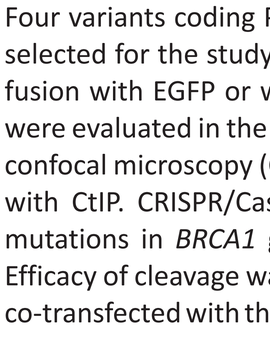

Figure 1: Schematic representation of transcriptional activation assay. (A) Represents the behavior of wild type protein; (B), a benigin polymorphism; (C), a missense variant (cancer associated) and (D), a nonsense variant (cancer associated).

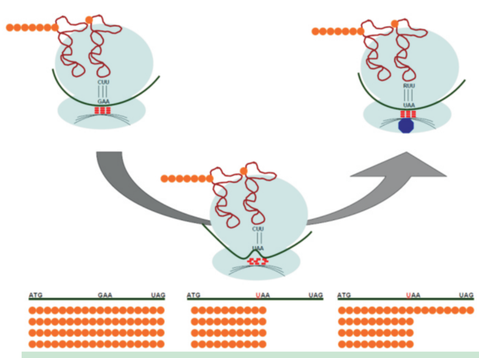

Figure 2: Schematic representation of aminoglycosides readthrough induction. The model illustrates the action of aminoglycosides on the ribosome during the translation process.

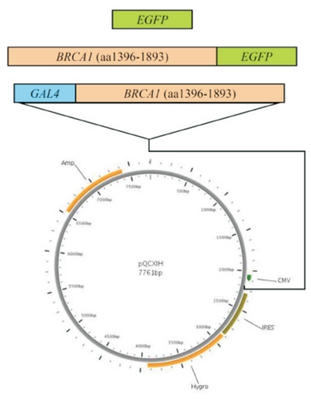

Figure 3: Clonning vector. The coding sequences for fusion proteins (EGFP in green, BRCA1 aa1396-1863 in orange and GAL4DBD in blue), including selected variants, were initially cloned using the TOPO ® cloning system. Subsequently, they were subcloned into pQCXIH

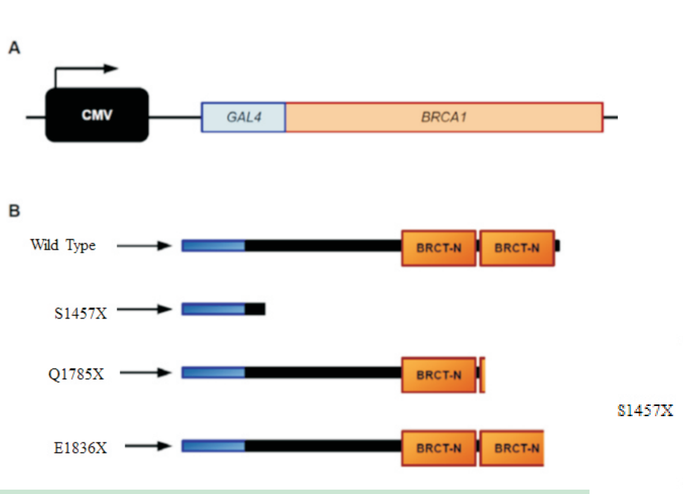

Figure 4: Schematic representation of GAL4:BRCA1 expression model. The promoter region (CMV) and *GAL4:BRCA1* DNA sequence were transducted in HeLa cells. Expression of these sequences result in different protein lengths according to the mutation position.

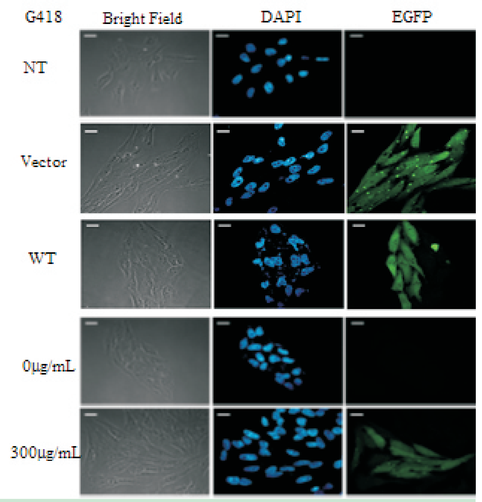

Figure 5: Readthrough analysis by confocal microscopy. The panel displays an experiment of induced readthrough (48 hours after treatment). Cells transiently transfected with wild type construct show a constitutive expression of BRCA1:EGFP. Cells transfected with *BRCA1* variant S1457X construct only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

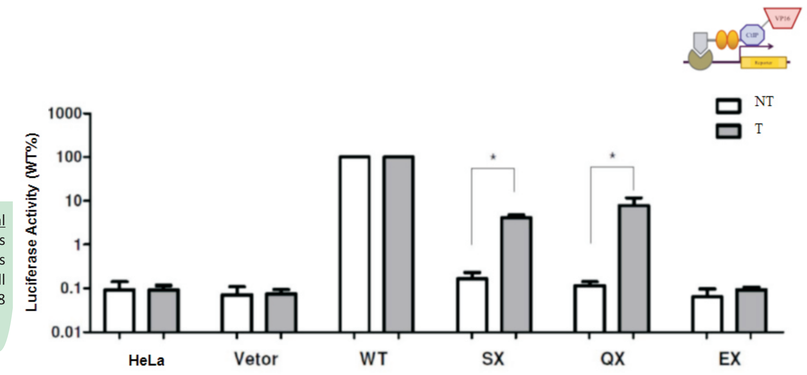

Figure 6: Readthrough analysis by transcriptional activation assay. Activity of wild type and variants GAL4:BRCA1 constructs was accessed by its interaction with CtIP fusioned with VP16. All variants showed very low activity without G418 and a increase in the activity after treatment (T).

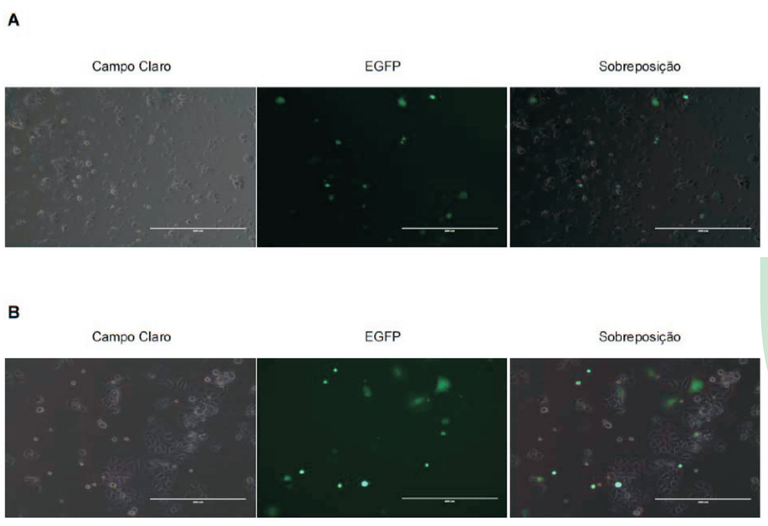

Figura 7: CRISPR/Cas9 genomic edition in MCF-7 cell line. The panel displays an experiment of genomic edition via CRISPR/Cas9 technology (24 hours (A) and 48 hours (B) after transfection). Cells transiently transfected with guide RNA SX1 construct and single strand donor DNA show a constitutive expression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

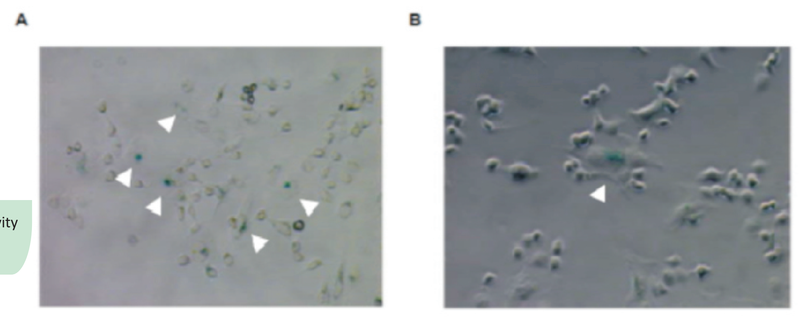

Figura 8: b-galactosidase assay. b-galactosidase activity was visualized in transiently cotransfected MCF-7 cells.

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

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