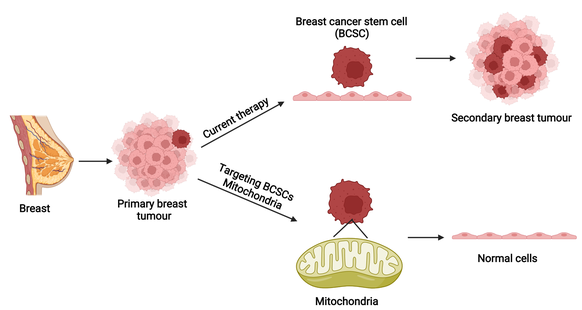
Role of Mitochondria in Breast Cancer

Chiara Chinigò [1], Michael Lisanti [1], Federica Sotgia [1]

[1] Translational Medicine, School of Science, Engineering and Environment (SEE), University of Salford, Greater Manchester, UK

BACKGROUND

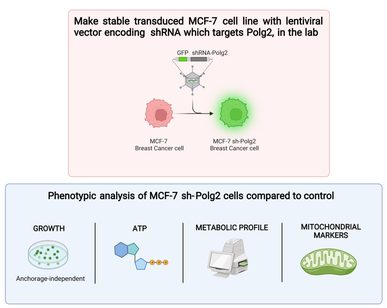


Cells within a tumour are not all the same but inside it, stronger and more malignant cells than others called "cancer stem cells" (CSCs) can be distinguished. Several studies demonstrate that mitochondrial metabolism represents the main energy source for breast cancer stem cells (BCSCs). For this reason, acting on mitochondria could be a breakthrough in targeted therapies against these malignant cells. In this context, the mitochondrial DNA polymerase γ, which is fundamental for mitochondrial homeostasis, could be considered as a potential mitochondrial target for anti-cancer therapy because its dysfunction may have a role in breast cancer progression.

AIM

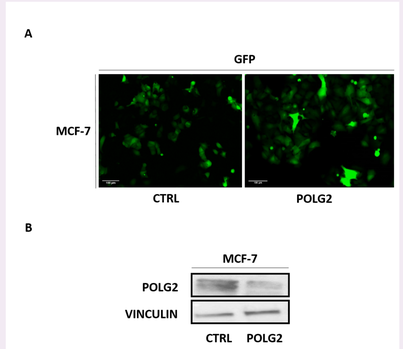
The overall aim of this project is to investigate the role of mitochondrial DNA polymerase γ in influencing the phenotype of breast cancer cells and to use this model system for drug screening, in search of drugs which may target the polymerase acting on mitochondrial metabolism, leading to inhibition of BCSCs dissemination.

METHODS

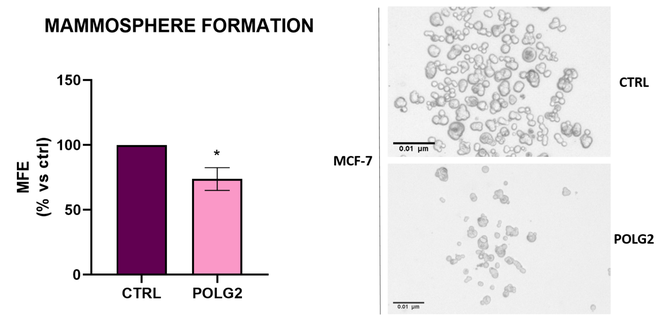


In order to investigate the role of Polg2 subunit of mitochondrial DNA polymerase γ in breast cancer an shRNA approach was used, generating stably-transduced MCF-7 cell line with a lentiviral vector encoding and shRNA which targets Polg2 and an empty vector as control.Then the analysis of the phenotype of MCF-7 transduced and control cells was performed.

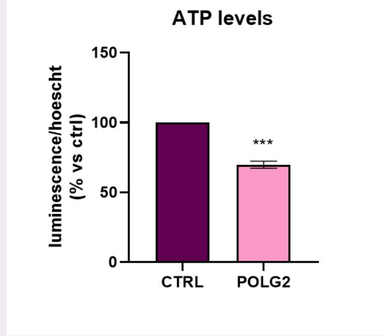
RESULTS



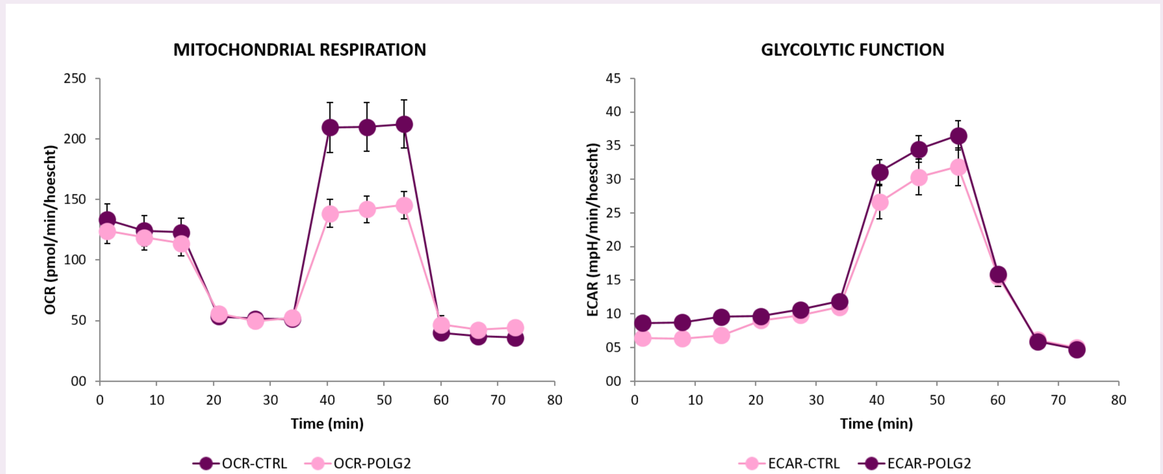
Knockdown of nuclear gene which codified for Polg2 subunit of mitochondrial DNA polymerase γ: A MCF-7 breast cancer cells were transduced with sh-RNA vectors targeting Polg 2 gene and the scrambled control; **B** Protein expression of Polg2 evaluated by immunoblotting performed on MCF-7 cells.



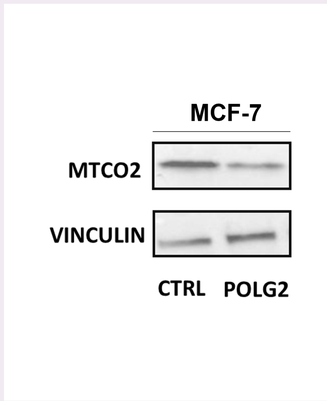
Polg2 knockdown determines a decrease in mammosphere formation: MCF-7 transduced cells and control cells were grown in low attachment conditions as tumour spheres. After 5 days spheres >50 µm were counted manually.



Polg2 knockdown diminishes ATP production: Evaluation of ATP levels in MCF-7 transduced cells and control cells by CellTiter-Glo. Luminescence was detected using Varioskan LUX plate reader.



Polg2 knockdown determines a decrease in mitochondrial respiration: The Seahorse XFe96 Analyzer was employed to determine the mitochondrial function and the status of glycolysis of MCF-7 transuced cells and control cells.



Polg2 knockdown determines a reduction of MT-CO2 protein levels: the cytochrome c oxidase subunit 2 is the second subunit of the complex IV of the respiratory chain and it is one of the three mt-DNA encoded protein of the complex IV. MT-CO2 levels were evaluated by Immunoblotting in MCF-7 transduced cells and control cells

CONCLUSION

In conclusion, our findings may have important implications for the clinical prevention of breast cancer progression, using a metabolism-based approach for targeting mitochondria.