Does the loss of BIG3 expression in Breast cancer contribute to brain metastasis?

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BRAIN TUMOUR NORTH WEST

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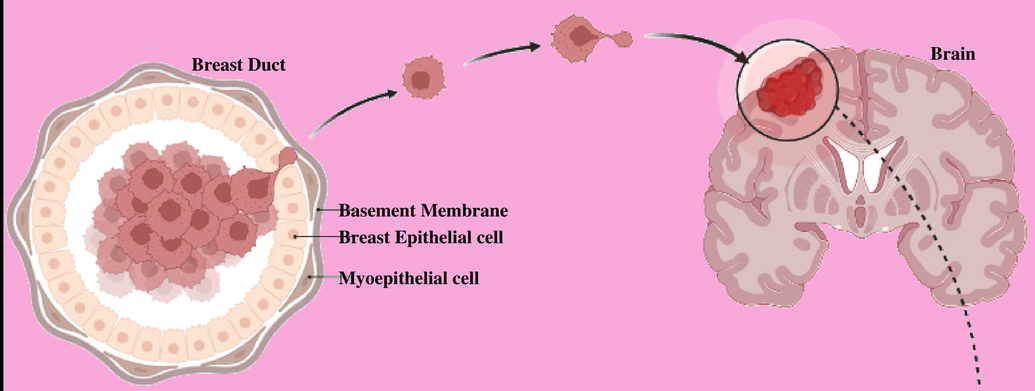
INTRODUCTION

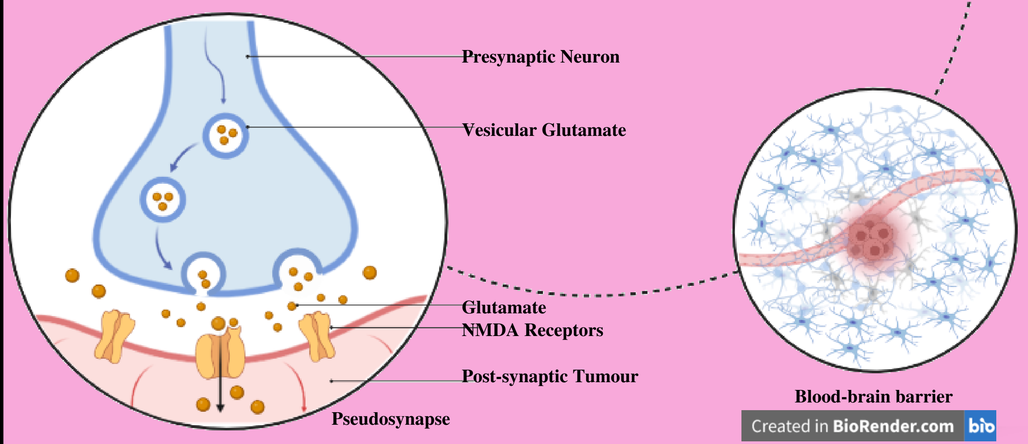
- Breast cancer is the most common cancer worldwide. In 2020, there were 2.3 million women diagnosed with breast cancer and 685,000 deaths globally. Breast cancer cases in the UK represent 15% of all new cancer cases. In 2022 it is estimated to cross 287,850 cases *(1)(4)*.
- ARFGEF3 (BIG3) is a guanyl-nucleotide exchange factor that regulates systemic glucose homeostasis, negatively regulating insulin granule biogenesis in pancreatic islet beta cells and inhibits the GABA signaling in neurons *(2)*.
- We have found that *BIG3* is frequently mutated in breast tumours that metastasize to the brain.
- It Inhibits nuclear translocation of the transcriptional coregulator PHB2 and may enhance estrogen receptor alpha (ESR1) transcriptional activity in breast cancer cells.
- Some breast cancer cells express neurotransmitter receptors which promote tumour growth in the brain. The activation of glutamate ligands of N-methyl-D-aspartate receptors (NMDARs) enables metastatic tumour cells to proliferate in the brain microenvironment *(3)*.

HYPOTHESIS

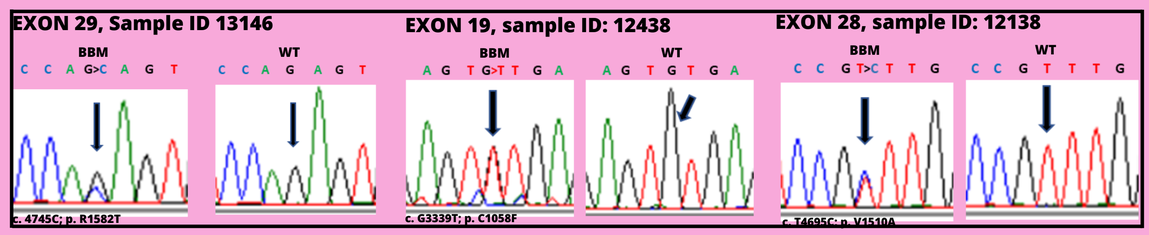
- Our hypothesis states that the loss of BIG3 in a breast cancer cell line contributes to brain metastasis via the increase in the expression of certain neurotransmitters and lysosomes (LAMP1), which in turn promotes migration and proliferation in the brain.



Cancers in the brain form pseudo-synapses which promotes tumour growth *(5)*

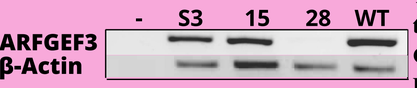


SEQUENCING



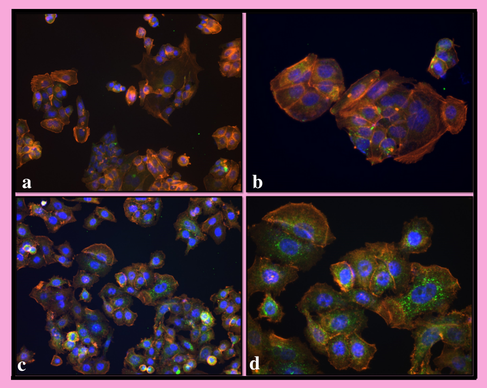
Sequencing was performed to identify brain-metastasis-specific mutations in ARFGEG3 (BIG3).

RT CONFIRMATION



To mimic BIG3 mutations seen in tumours we knocked it out by CRISPR. Clone 28 showed complete BIG3 silencing (S3= scrambled control).

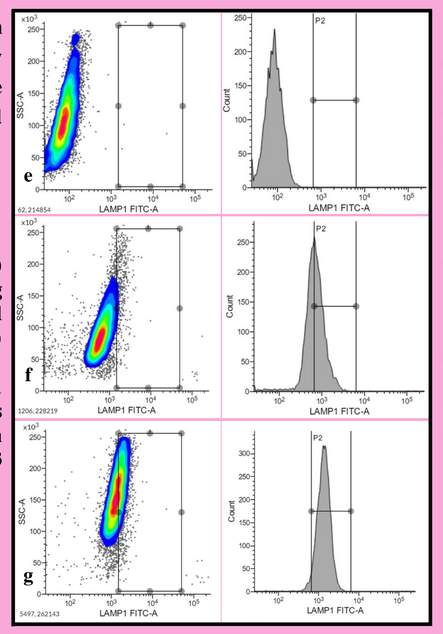
IMMUNOSTAINING



LAMP 1 (lysosome marker) expression in Big3 expressing MCF7 cell line (a, b) and BIG3 Knockout clone 28 (c, d)

Upregulation in LAMP 1 expression (Green) was observed in the knockdown clone when compared to BIG3 expressing cells (S3).

FLOW CYTOMETRY

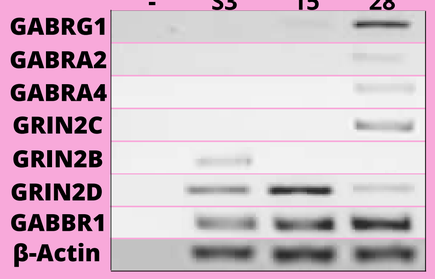


Quantitative analysis of LAMP1 expression by Flow Cytometry.
(e) non-staind cells
(f) BIG3 expressing cells (S3)
(g)BIG3 knock-down cells (K.D28)

A distinct shift in the peak was observed in K.D 28 with respect to S3 indicating the increase in LAMP1 expression in MCF7 cell line when BIG3 was knocked down. These results confirm the Immunostaing results and suggests that BIG3 loss is associated with lysosomal upregulation.

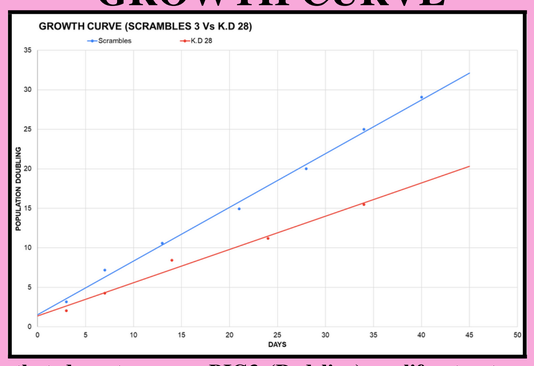
Lysosome activity is associated with neurotransmitter receptor modulation *(2)*.

NEUROTRANSMITTERS EXPRESSION



Expression analysis of various neurotransmitters in MCF7 cell line differentially expressing BIG3. We observed an upregulation of some neurotransmitters in the BIG3 knock-down cells (K.D 28) compared to BIG3 expressing cells to (S3). both gabaergic receptors and NMDA receptors were differentially expressed. This modulation in neurotransmitter receptors may contribute to pseudo-synapse formation.

GROWTH CURVE



MCF7 cells that do not express BIG3 (Red line) proliferate at a slower rate than control cells expressing BIG3 (Blue line). This reduced proliferation may be as a consequence of significant cell physiological changes associated with BIG3 loss. Slow proliferation may contribute to increased tumour survival; *in vivo* studies will be required to further investigate this.

REFERENCES

(**1**) *Breast cancer statistics*. Cancer Research UK (2022). https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer

(2) Liu, T., Li, H., Hong, W., & Han, W. (2016). Brefeldin A-inhibited guanine nucleotide exchange protein 3 is localized in lysosomes and regulates GABA signaling in hippocampal neurons. Journal of neurochemistry, 139(5), 748–756. https://doi.org/10.1111/jnc.13859

(3) Zeng, Q., Michael, I., Zhang, P., Saghafinia, S., Knott, G., Jiao, W., McCabe, B., Galván, J., Robinson, H., Zlobec, I., Ciriello, G. and Hanahan, D., 2019. Synaptic proximity enables NMDAR signalling to promote brain metastasis. Nature, 573(7775), pp.526-531.

(4) National Breast Cancer Coalition. 2022. Breast Cancer Statistics | Facts & Figures | NBCC. [online] Available at: <https://www.stopbreastcancer.org/information-center/facts-figures/> [Accessed 9 June 2022].

(5) Venkatesh, H.S., Morishita, W., Geraghty, A.C. et al. Electrical and synaptic integration of glioma into neural circuits. Nature 573, 539–545 (2019). https://doi.org/10.1038/s41586-019-1563-y

(6) BioRender. 2022. BioRender. [online] Available at: <https://biorender.com/> [Accessed 9 June 2022].