Effect of NMDA Receptor Antagonist in the Progression of Inflammatory Breast Cancer

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Introduction

- It is estimated that more than 1.9 million new cases of cancer will be diagnosed in 2022. Breast cancer is the most common cancer among females (31%). Inflammatory Breast Cancer (IBC) is rare and accounts for only 1% to 5% of all breast cancers and it doesn't look like a typical breast cancer. It often does not cause a breast lump, and it might not show up on a mammogram and this makes it harder to diagnose but has a 73% incidence of metastasis to the brain as compared to other cancer types.
- Extracellular signal-regulated kinase 1/2 (ERK) belongs to the mitogen-activated protein kinase (MAPK) family, which plays a role in signaling cascades and transmits extracellular signals to intracellular targets. Due to the importance of the ERK cascade, ERK disorders are harmful to cells and ultimately to the body. Excessive activation of upstream proteins and kinases in the ERK pathway has been shown to induce various diseases, including cancer.
- PI3K and AKT network pathway adds two major hallmarks of cancer: growth factor independence through oncogenic signaling and metabolic reprogramming to support cell survival and proliferation.

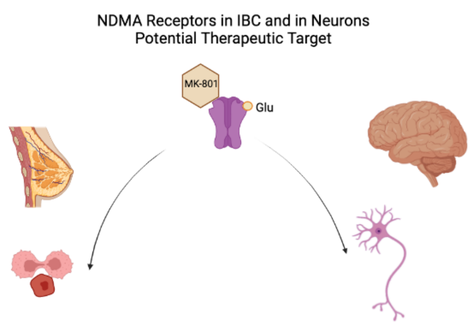

Fig. 1 NMDA receptors (NMDARs) constitute important calcium channels that are chiefly found within the central nervous system. Overstimulation of the calcium channel results in cell death. Antagonists of this receptor, such as dizocilpine maleate (MK-801), have been developed and used as a form of therapy for Alzheimer's disease by irreversibly blocking the excessive calcium influx through these channels. This functional receptor is expressed by inflammatory breast cancer. Targeting this receptor with the small-molecule antagonists MK-801 significantly decreases in vitro cell viability. Findings indicate NMDARs are critical for cancer cell growth and can be used as a potential target for successful cancer therapy.

Objective and Rationale

The purpose of this work is to study the effect of the neuropsychiatric drug MK-801 as antagonist of NMDARs blocking its Ca^{2+} mediated signaling. IBC shows overexpression of NMDARs. If ERK cascade and PI3K-AKT network require calcium ion for the phosphorylation, then the expression of pERK1/2 and pAKT should be affected. Also is important to consider the presence of glutamate in the in the media because it is needed by the NMDA receptor. The results may contribute to elucidate role of the receptor and to explore an alternative target for prevention of breast to brain metastases.

Methodology

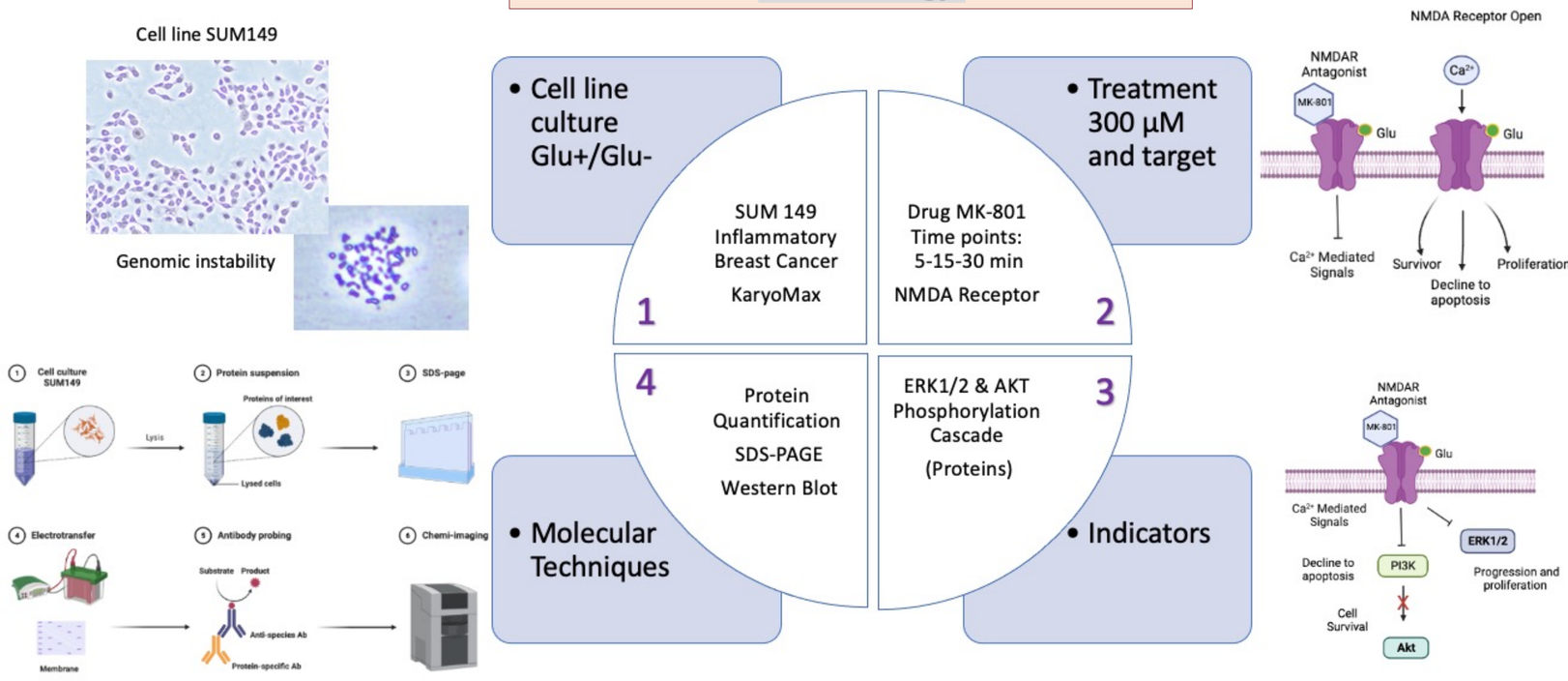

Fig. 2 These general research skills and molecular techniques will be adapted for high school students as part of the BIORET 2022 ***Research in Action.*** The goal is to take informed decisions about how to improve teaching and learning based on evidence. A systematic study of the action can help to visualize its impact on STEM education offered in a boarding school specialized in Science and Mathematics.

Results

Protein	SUM149 Glu-	SUM149 Glu+
pERK		
Total ERK		
pAKT		
Total AKT		
GAPDH		

Fig. 3 Normalization of Western blotting was performed with Total ERK1/2, Total AKT and GAPDH.

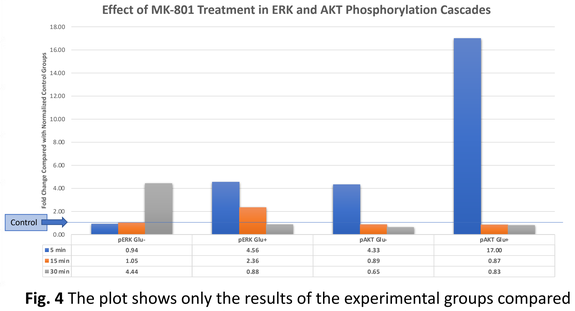

Fig. 4 The plot shows only the results of the experimental groups compared with the normalized control groups represented by the line across the bars.

- Our data suggest that both pathways were affected by the time of treatment and the absence of glutamate in the media.
- By contrast with the other conditions, the expression of pERK showed an increased fold about 4.4X at 30 min of treatment without glutamate. The tendency of the other conditions was to decrease the the expression of the phosphorylated proteins of interest while the time of treatment was increased.
- The expression of pERK and pAKT at 5 min of treatment in the presence of glutamate is near 4x greater than the cultures without glutamate.
- The most remarkable finding was in the relative expression of pAKT after the second and third time points because it decreased and kept lower than control samples independently of glutamate in media. This may represent an interesting target of study because if cell survival can be affected by the drug, then elucidating the mechanism can lead an alternative pharmacological treatment for prevention of brain metastases.

Conclusion

We evaluated the sensitivity of ERK1/2 and PI3K-AKT signaling pathways in IBC cell cultures exposed to NMDAR noncompetitive antagonists with and without glutamate. Two findings emerge from this study. First, these data showed that Ca^{2+} mediated signals like ERK1/2 and PI3K-AKT are sensitive at different time points to MK-801 and to the absence of glutamate in media. Second, the underexpression of pAKT independently of glutamate has the potential therapeutic relevance that opens a possible target to be explore in future studies associated with blocking cell survival.

Acknowledgement

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