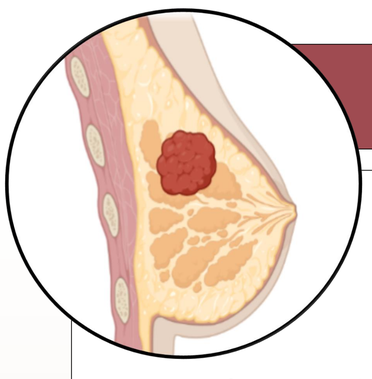
Investigating the effect of HIFs and hypoxia on VGSCs in triple-negative breast cancer cells

NATTANAN SAJJABOONTAWEE[1,2], KATHERINE S. BRIDGE[1,2], WILLIAM J. BRACKENBURY[1,2]

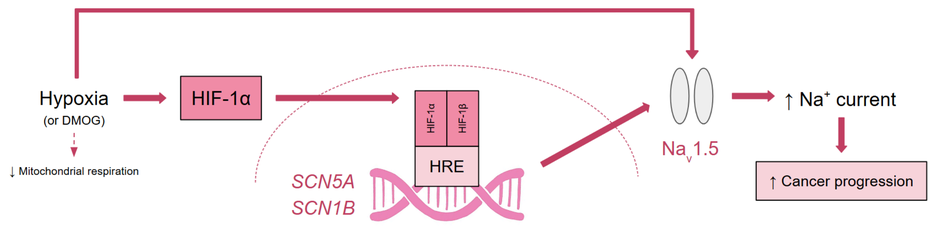
[1]Department of Biology, University of York, York, UK
[2]York Biomedical Research Institute, University of York, York, UK



INTRODUCTION

- **Triple-negative breast cancer (TNBC):** Lacks hormone receptors, more aggressive and challenging to treat compared to ER+ breast cancer[1].
- **Voltage-gated Na^+ channels (VGSCs):** α subunit and auxiliary β subunits[2]
  - **$Na_v1.5$ (*SCN5A*):** Up-regulated in TNBC, promotes invasion and metastasis[3].
  - **β1** (***SCN1B***): Controls expression and activity of $Na_v1.5$, cell adhesion molecules[4].
- **Hypoxia:** Low O_2 in tissues, usually developed in breast tumours
  - Hypoxia increases the persistent current of $Na_v1.5$ in the ischaemic heart via SUMOylation and causes an increase in intracellular $[Na^+]$ in breast cancer[6,7].
- **Hypoxia-inducible factors (HIFs):** Key regulators of the cellular hypoxic response
  - HIF-1α is the most critical subunit, overexpressed in breast cancer and significantly enhances metastasis[8].

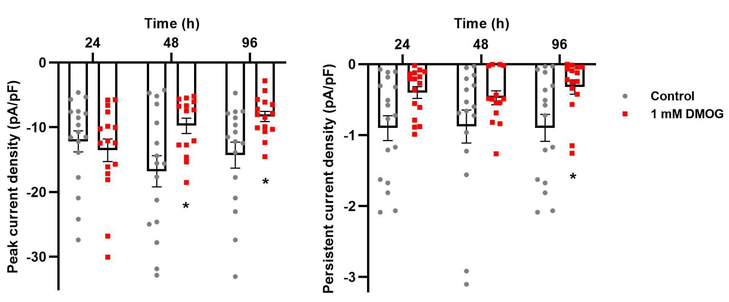
Hypothesis: HIF-1α is responsible for the upregulation of $Na_v1.5$ in hypoxia, and hypoxia can alter $Na_v1.5$ function post-transcriptionally. These lead to higher channel activity and increased invasive properties of breast cancer.



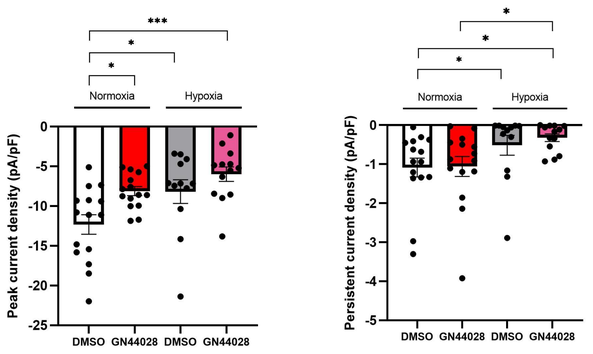
RESULTS

Effect of DMOG and hypoxia on Na^+ currents

- **Electrophysiology:** Peak current and persistent current decreased in MDA-MB-231 cells treated with 1 mM dimethyloxalylglycine (DMOG), a HIF stabiliser and α-ketoglutarate analogue.

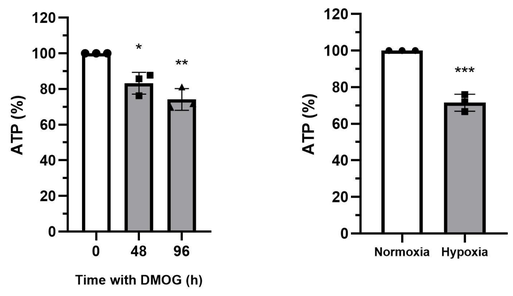


- Peak current and persistent current also decreased in cells cultured in hypoxia (1% O_2) for 48 hours.
- 1 μM GN44028, a HIF-1α inhibitor, did not suppress the inhibiting effect of hypoxia on Na^+ currents.

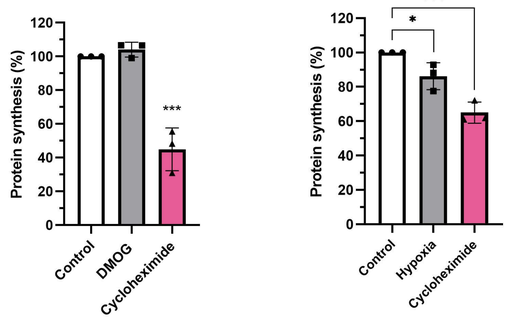


Effect of DMOG and hypoxia on metabolism

- **CellTiter-Glo® assay:** ATP levels decreased in cells treated with DMOG or hypoxia for 48 or 96 hours.

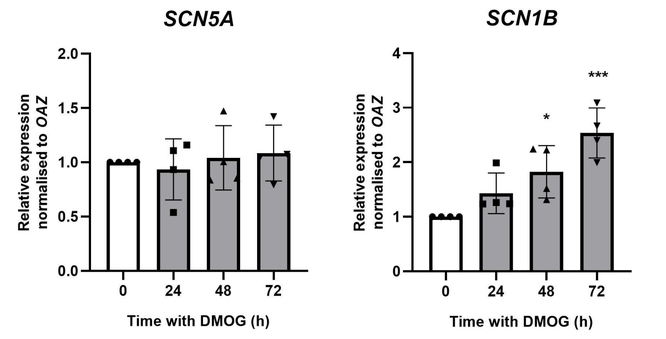


- **Protein synthesis assay:** Protein synthesis decreased in cells cultured in hypoxia for 48 hours.

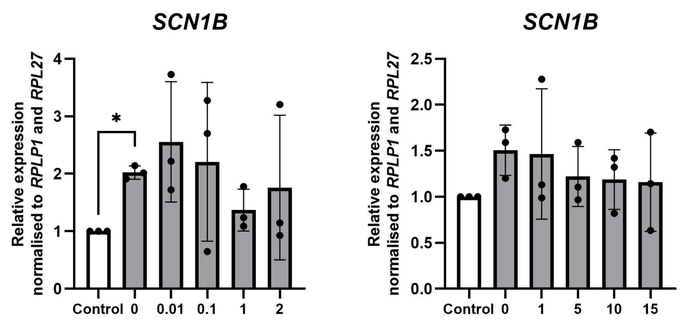


Effect of HIF stabilisation and inhibition on *SCN5A* and *SCN1B* expression

- **rt-qPCR:** Increased expression of the canonical HIF target genes (*GLUT1* and *CA9*) showed that DMOG successfully stabilised HIFs in the MDA-MB-231 cell line.
- *SCN5A* expression was not affected by DMOG at 24, 48, or 72 hours of DMOG incubation.
- *SCN1B* expression was upregulated in DMOG-treated cells in a time-dependent manner.

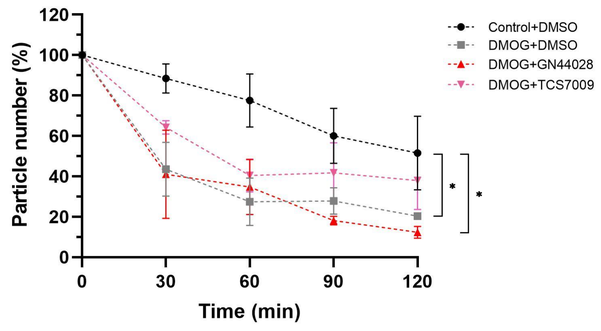


- Inhibiting HIF-1α by GN44028 may suppress the upregulation of *SCN1B* by DMOG, but the effect was not significant.
- Inhibiting HIF-2α by TCS7009 did not suppress the effect of DMOG on *SCN1B* expression.



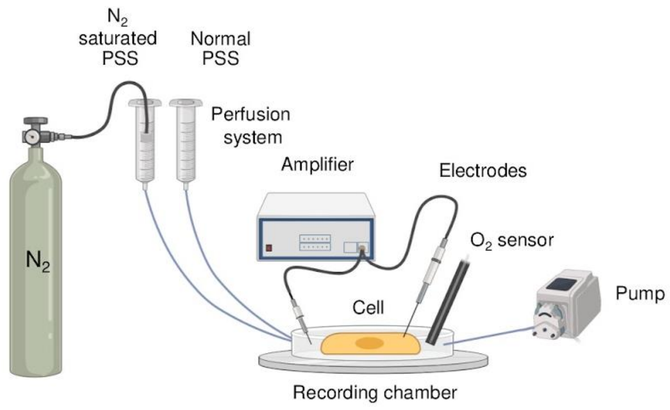
Effect of DMOG on cell adhesion

- **Cell-cell adhesion:** Cell adhesion increased in cells treated with DMOG after 72 hours of treatment.
- 5 μM TCS7009 may suppress the effect of DMOG on cell adhesion.

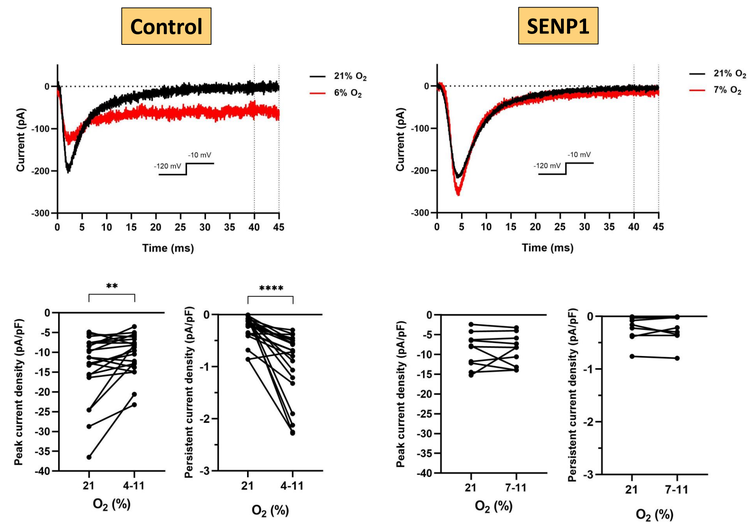


Effect of acute hypoxia on Na^+ currents

- **Hypoxic patch clamp system**



- Acute hypoxia (4-11% O_2) decreased peak current and increased persistent current in MDA-MB-231 cells.
- There was a significant negative correlation between O_2 levels and persistent current.
- A deSUMOylase, SENP1 (1 nM), inhibited the effect of acute hypoxia on VGSC currents.



CONCLUSION

In MDA-MB-231 cells, DMOG and chronic hypoxia pre-incubation decreased Na^+ currents possibly by inhibiting the formation of the $Na_v1.5$ channels as a result of metabolic suppression while acute hypoxia increased persistent currents possibly via SUMOylation of $Na_v1.5$. Further work is required to delineate the mechanisms. HIF stabilisation using DMOG did not alter *SCN5A* expression but increased *SCN1B* expression, which might be responsible for the increase in cell adhesion caused by DMOG. HIFs may not have a direct effect on $Na_v1.5$ expression and activity.

References:

1. Hartkopf, A. D., Grischke, E.-M. & Brucker, S. Y. Endocrine-Resistant Breast Cancer: Mechanisms and Treatment. Breast Care 15, 347–354 (2020).
2. Catterall, W.A. Structure and function of voltage-gated sodium channels at atomic resolution. Exp Physiol. 99, 35–51 (2014).
3. Nelson, M., Yang, M., Millican-Slater, R. & Brackenbury, W. J. Nav1.5 regulates breast tumor growth and metastatic dissemination in vivo. Oncotarget 6, 32914–32929 (2015).
4. Chioni, A.-M., Brackenbury, W. J., Calhoun, J. D., Isom, L. L. & Djamgoz, M. B. A. A novel adhesion molecule in human breast cancer cells: voltage-gated Na+ channel beta1 subunit. Int. J. Biochem. Cell Biol. 41, 1216–1227 (2009).
5. Semenza, G. L. The hypoxic tumor microenvironment: A driving force for breast cancer progression. Biochim. Biophys. Acta 1863, 382–391 (2016).
6. Plant, L. D., Xiong, D., Romero, J., Dai, H. & Goldstein, S. A. N. Hypoxia produces pro-arrhythmic late sodium current in cardiac myocytes by SUMOylation of NaV1.5 channels. Cell Rep. 30, 2225–2236.e4 (2020).
7. Djamgoz M.B.A., Fraser S.P., Brackenbury W.J. In vivo evidence for voltage-gated sodium channel expression in carcinomas and potentiation of metastasis. Cancers [Internet]. 11 (2019).
8. Liao, D., Corle, C., Seagroves, T. N. & Johnson, R. S. Hypoxia-inducible factor-1alpha is a key regulator of metastasis in a transgenic model of cancer initiation and progression. Cancer Res. 67, 563–572 (2007).