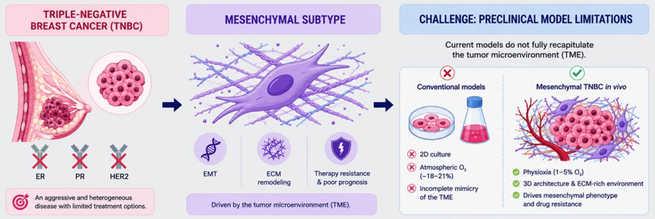
Modelling the Mesenchymal Breast Cancer Microenvironment Using Low-Shear 3D Culture and Physioxia

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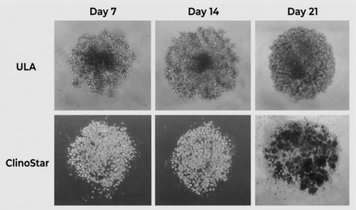
Introduction

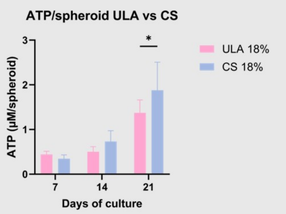
Triple-negative breast cancer (TNBC) is an aggressive and heterogeneous disease with limited treatment options. The mesenchymal subtype is characterized by epithelial-to-mesenchymal transition (EMT), extracellular matrix (ECM) remodeling, enhanced invasiveness, and therapy resistance. Understanding this subtype remains challenging due to the lack of preclinical models that accurately recapitulate the tumor microenvironment and its physiological conditions.

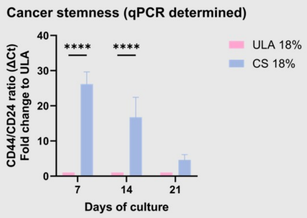


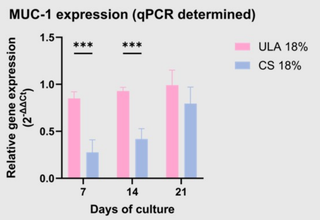
Low-shear culture enhances viability and stem-like features

Dynamic culture in the ClinoStar® (CS) significantly improved spheroid viability and long-term maintenance compared with static ULA culture. ClinoStar-cultured spheroids also displayed an increased CD44/CD24 ratio at Days 7 and 14, indicative of enhanced cancer stem-like characteristics. In contrast, MUC1 expression was elevated in static cultures, suggesting a more epithelial-associated phenotype.

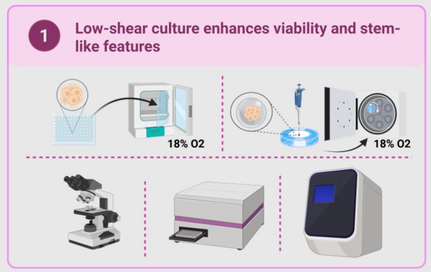


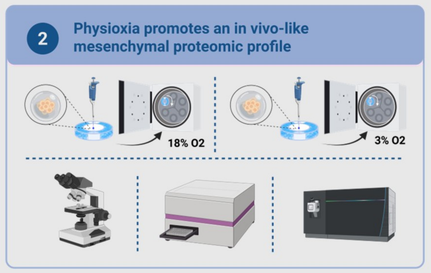


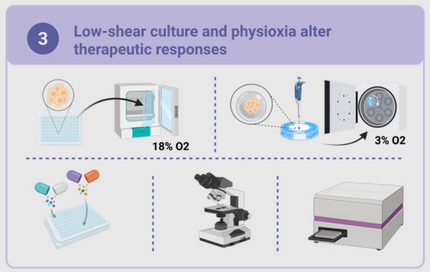




Experimental setup & workflow

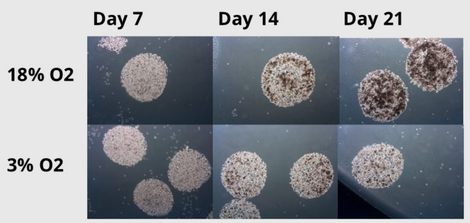


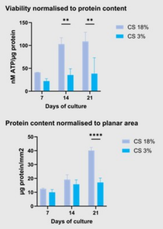


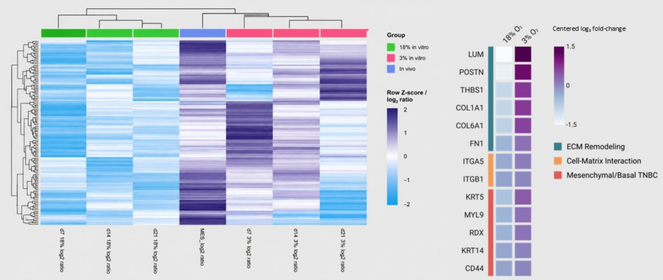


Physioxia promotes an in vivo-like mesenchymal proteomic profile

MDA-MB-231 spheroids cultured under atmospheric oxygen (18% O_2) displayed increased growth, ATP production, and total protein content compared with spheroids maintained under physioxia (3% O_2). Despite this enhanced proliferative phenotype, hierarchical clustering of Day 14 proteomic profiles revealed that 3% O_2 cultures clustered more closely with in vivo tumor samples, whereas 18% O_2 cultures formed a distinct group. Furthermore, physioxia enriched proteins associated with extracellular matrix remodeling (LUM, POSTN, THBS1, COL1A1, COL6A1, FN1), cell–matrix interactions (ITGA5, ITGB1), and mesenchymal/basal TNBC features (KRT5, CD44, MYL9, RDX), consistent with characteristics reported for mesenchymal TNBC.

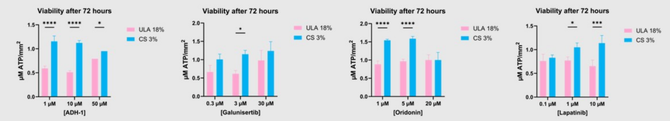






Low-shear culture and physioxia alter therapeutic responses

ClinoStar® continuous laminar fall conditions ensure low-shear conditions for the cultured spheroids. Following 72 h drug exposure, ClinoStar-cultured spheroids maintained higher ATP levels than ULA-cultured spheroids across multiple compounds and concentrations. The largest differences were observed for ADH-1, Oridonin, and Lapatinib, suggesting enhanced preservation of treatment-resistant features associated with mesenchymal TNBC.



Conclusions

Low-shear culture and physioxia promote stem-like, mesenchymal, and extracellular matrix remodeling features in MDA-MB-231 spheroids. Despite reduced proliferation, physioxia generated a proteomic profile more closely resembling in vivo tumors and altered therapeutic responses. Together, these conditions can improve the physiological relevance of in vitro mesenchymal TNBC models.

Acknowledgements

Proteomics analyses were performed at the University of Southern Denmark (SDU). Support from the Novo Nordisk Foundation (INTEGRA, NNF20OC0061575) and the Danish Agency for Higher Education and Science (PLATO, 5229-00012B) is gratefully acknowledged.

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