RESEARCH HIGHLIGHT

Banking on metabolomics for novel therapies in TNBC

Aurelien Tripp[1] and George Poulogiannis[1]✉

Cell Research (2022) 32:423–424; https://doi.org/10.1038/s41422-022-00637-7

Despite sharing the lack of hormone receptors and HER2 amplification used to guide therapeutic decisions, Triple-Negative Breast Cancer (TNBC) is a highly heterogeneous subset of breast cancer. In a recent study published in *Cell Research*, Xiao et al. highlight the opportunity of using this tumor subtype's metabolic signatures to better stratify patients and identify more effective therapeutic strategies in TNBC.

Patients with breast cancer (BC) staining positive for the expression of estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2) benefit from targeted therapies, as first-line treatment options. However, Triple-Negative Breast Cancer (TNBC), accounting for 15%–20% of BC cases,[1] and characterized by not expressing hormone receptors or bearing HER2 amplification in general lacked targeted therapy approaches. Minor exceptions include approved usage of Olaparib and Talazoparib, or an immune checkpoint inhibitor, Atezolizumab for patients with germline mutation in *BRCA* genes and PD-L1$^+$ tumors, respectively. While many others are under investigation, such as antibody–drug conjugates and PI3K/AKT pathway inhibitors,[2] the shift towards precision medicine means that novel therapies only benefit a subset of patients. This highlights a major unmet clinical need for establishing biomarkers to better stratify TNBC that will ultimately help with identifying novel targets for more tailored and effective therapeutic options.

Advances in high-throughput molecular techniques have provided new insights that TNBCs are highly heterogeneous. There are multiple existing classifications, the majority of which rely on genomic and transcriptomic data, but the singularity of each of these approaches lies in the selected method to stratify the disease. In 2011, Lehmann et al.[3] initially identified six subtypes by combining gene expression from 21 datasets and performing k-mean clustering, and later refined these to 4. Based on a study by Jezequel et al,[4] TNBC could be divided in 3 clusters, by applying fuzzy clustering on gene expression of an internal cohort. Following a more focused approach, by selecting specific gene expression signatures, TNBC was shown to be classified into three subtypes based on immune-related genes[5] or three subtypes based on metabolism-related genes.[6] Interestingly, Jiang et al.[7] stratified TNBC in four subtypes based on transcriptomic data: (1) luminal androgen receptor (LAR), (2) immunomodulatory (IM), (3) basal-like immune-suppressed (BLIS), and (4) mesenchymal-like (MES), showing significant overlap with Lehmann's stratification. This led to the initiation of the FUTURE clinical trial (NCT03805399)[8] with satisfactory response for two out of the four subtypes (IM and MES). However, therapeutic options for the other two subtypes (BLIS and LAR) did not meet expectations and alternative approaches are deemed necessary.

Among the new OMICS technologies, one that has lately gained considerable interest is metabolomics. This technology allows for measurement of abundance of metabolites, but more importantly, enables the study of metabolic reprogramming observed in cancer and other diseases.[9] This could pave the way towards the discovery of metabolites being used as biomarkers for disease stratification and therapy response, and metabolism-based therapeutic options.

In their study published in *Cell Research*, Xiao et al.[10] followed up on their previous stratification[7] to look for metabolic reprogramming events occurring in specific TNBC subtypes, with a special interest in identifying novel therapeutic avenues for the LAR and BLIS subtypes. To this end, the team profiled a subset of patients with TNBC using Liquid Chromatography–Mass Spectrometry (LC–MS) to investigate over 2500 polar metabolites and lipids, making it one of the largest studies both in the number of samples and metabolites being analyzed. Clustering using similarity network fusion method revealed that TNBC can be divided in three subgroups based on their metabolic signatures, each having enrichment in the abundance of specific groups of metabolites. The C1 group was characterized by an increase in lipid species and accordingly, was more reliant on fatty acid metabolism to meet their energetic needs. The C2 group exhibited higher levels of species involved in carbohydrate and oxidation pathways and was predicted to be more reliant on glutamate metabolism, whilst the C3 group showed fewer overall differences compared to normal tissue.

To test the significance of this new classification, the authors investigated its potential association with the previously described transcriptomic subtypes[7] and the relapse status of TNBC patients. A significant correlation was highlighted between the metabolic C1 and transcriptomic LAR subtypes, whilst the association for C2 and C3 with the remaining three transcriptomic subtypes was less clear, with the tumors classified in these metabolic subtypes equally being distributed among the remaining transcriptomic subtypes. It was however established that the portion of BLIS tumors overlapping with the C2 metabolic subtype had overall worse relapse-free survival. Using two machine learning methods, namely LASSO and SVM, it was however possible to distinguish between BLIS-C2 and BLIS-C3 tumors based on the abundance of as little as 6 metabolites. The establishment of strong links between metabolomic and transcriptomic subtypes suggested a potential for novel therapeutic avenues in the LAR and BLIS groups that showed poor response in the FUTURE trial.

[1]Signalling and Cancer Metabolism Team, Division of Cancer Biology, The Institute of Cancer Research, London, UK. ✉email: george.poulogiannis@icr.ac.uk

Published online: 28 February 2022

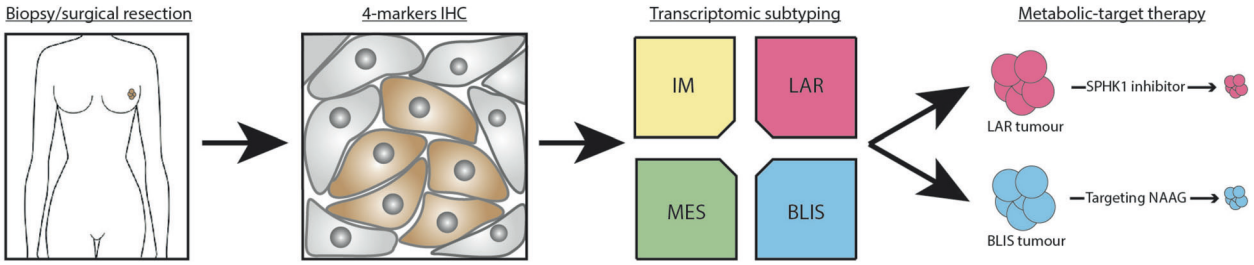

Fig. 1 A new pipeline for targeted therapies for treatment-refractory TNBCs. Work from Xiao et al. could suggest a new pipeline integrating multiple approaches to treat patients with TNBC. Following biopsy or surgical resection of a tumor, immunohistochemistry (IHC) using a panel of four markers could stratify patients in one of four transcriptome-based subtypes. Targeting metabolic vulnerabilities specific to treatment-refractory TNBC subtypes could enable individual-tailored treatment regimens.

Identification of functional metabolites being enriched in transcriptomic subtypes could highlight dependencies to metabolic pathways that are clinically exploitable.

Comparison of lipid profiles from LAR versus non-LAR tumors also showed increased levels of ceramides in the LAR group, with this phenotype getting validated using tracing experiments that confirmed increased de novo synthesis and degradation of ceramides in LAR tumors, consistent with the observation that the C1 subtype might be more reliant on fatty acid metabolism. To test for potential vulnerabilities, the authors turned to preclinical models, including patient-derived organoids and patient-derived xenografts to show that SPHK1 inhibitors were more effective in LAR compared to non-LAR models, stressing the importance of this targetable metabolic vulnerability in the transcriptomic LAR subtype.

Using a similar approach for BLIS-C2 tumors and restraining the selection of metabolites only to those associated with poor prognosis, the authors identified *N*-acetyl-aspartyl-glutamic acid (NAAG) as a good candidate for further follow-up work. Production of NAAG is attributed to two enzymes, but only RIMKLB was shown to be highly expressed in BLIS tumors, whilst its expression level was also positively correlated with NAAG levels both in the TNBC tumor and cell line cohort. Notably, shRNA-mediated inhibition of RIMKLB in BLIS preclinical models led to a significant reduction in cancer cell proliferation, migration, and invasion both in vitro and in vivo, but this effect could be rescued following supplementation with NAAG. Targeting this oncometabolite biosynthesis in BLIS tumors could therefore be an attractive therapeutic strategy for an otherwise difficult-to-treat tumor subtype.

Advances in OMIC technologies has led to multiple stratifications of TNBC, but clinical applications remain challenging. Potential issues lie in the integration of datasets coming from various methods and origins, but also the need for a fast and cost-effective method for tumor classification. This study adds a new brick to a multilayered approach aiming to develop targeted therapies suitable for each patient with TNBC. Hitting a roadblock for LAR and BLIS tumors in the FUTURE trial, Xiao et al. turned to metabolomics to find alternative options. By combining genomic, transcriptomic, metabolomic and immunohistochemistry, this study unveils novel metabolic vulnerabilities and highlights a potential pipeline for precision medicine across treatment-refractory TNBC subtypes (Fig. 1).

REFERENCES

1. Denkert, C. et al. *Lancet* **389**, 2430–2442 (2017).
2. Lyons, T. G. *Curr. Treat. Options Oncol.* **20**, 82 (2019).
3. Lehmann, B. D. et al. *J. Clin. Invest.* **121**, 2750–2767 (2011).
4. Jezequel, P. et al. *Breast Cancer Res.* **21**, 65 (2019).
5. He, Y. et al. *J. Exp. Clin. Cancer Res.* **37**, 327 (2018).
6. Gong, Y. et al. *Cell Metab.* **33**, 51–64.e9 (2021).
7. Jiang, Y. Z. et al. *Cancer Cell* **35**, 428–440.e5 (2019).
8. Jiang, Y. Z. et al. *Cell Res.* **31**, 178–186 (2021).
9. Clish, C. B. *Cold Spring Harb. Mol. Case Stud.* **1**, a000588 (2015).
10. Xiao, Y. *Cell Res.* https://doi.org/10.1038/s41422-022-00614-0 (2022).

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to George Poulogiannis.