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Targeting the polyamine pathway—"a means" to overcome chemoresistance in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is characterized by its aggressive biology, early metastatic spread, and poor survival outcomes. TNBC lacks expression of the targetable receptors found in other breast cancer subtypes, mandating use of cytotoxic chemotherapy. However, resistance to chemotherapy is a significant problem, encountered in about two-thirds of TNBC patients, and new strategies are needed to mitigate resistance. In this issue of the *Journal of Biological Chemistry*, Geck *et al.* report that TNBC cells are highly sensitive to inhibition of the *de novo* polyamine synthesis pathway and that inhibition of this pathway sensitizes cells to TNBC-relevant chemotherapy, uncovering new opportunities for addressing chemoresistance.

TNBC,[2] which is defined by the *absence* of clinically actionable levels of estrogen, progesterone, and HER2 receptor expression, represents ~15–20% of all breast cancers, but is disproportionately responsible for breast cancer deaths, particularly in young women and women of African ancestry (1). Recent developments have expanded treatment options for some, but not all, TNBC patients. These include pharmacological inhibitors of poly(ADP-ribose) polymerase, for patients with germline BRCA mutations, and the programmed death-ligand 1 (PD-L1) inhibitor atezolizumab, in combination with nab-paclitaxel, for PD-L1–positive TNBC patients. Despite these advances, cytotoxic chemotherapy agents, such as doxorubicin (Dox) and cisplatin (CsP), persist as the only option for most TNBC patients (2). Response to chemotherapy is high-stakes for these patients, given both their fewer options and the strong correlation between response and survival (3). Notably, survival for the approximately one-third of TNBC patients who *do respond* to chemotherapy is comparable with the extended survival achieved by non-TNBC patients (4), highlighting the dire need for approaches that allow patients to shift from non-responders to responders. Given that previous evidence shows that increased polyamine synthesis promotes tumor initiation and growth, the authors sought to answer whether it could be targeted to increase TNBC sensitivity to chemotherapy.

Polyamines are aliphatic cations that are essential for normal cell function and have been implicated in diverse processes, including stabilization of chromatin structure and regulation of transcription factors and ion channels (5). The polyamines in mammalian cells include putrescine, derived from the amino acid arginine following its conversion to ornithine, and the higher-order polyamines spermidine and spermine. In healthy cells, the polyamine pool is maintained within a narrow physiological range through feedback-mediated inhibition of biosynthetic enzymes, stimulation of catabolic enzymes, and fine tuning of polyamine uptake and efflux, through a still poorly understood transport system(s). This exquisite calibration is lost in cancer cells, which have long been appreciated to have elevated polyamine levels and dysregulated expression of key players that normally balance the polyamine pool (6, 7). The link to cancer was further solidified when ornithine decarboxylase (*ODC*), one of two rate-limiting enzymes in the polyamine biosynthetic pathway, was shown to be a direct transcriptional target of the *Myc* oncogene (8). It is now known that various oncogenic pathways, including Ras and PI3K, impinge upon polyamine metabolism, promoting polyamine accumulation in cancer cells (7). Moreover, previous observations have shown that the depletion of the intracellular polyamine pool induces cell cycle arrest, which increases the DNA damage done by chemotherapeutics. Hence, a significant focus of the polyamine field over the past 5 decades has been the realization of its promise as a therapeutic target for cancer, which has remained frustratingly elusive, save promising studies in neuroblastoma (7) (RRID:SCR_002309). The present study by Geck *et al.* (9) brings this focus to bear on TNBC and asks whether inhibition of *de novo* polyamine synthesis may provide a means to mitigate chemoresistance.

Earlier studies reported that, like other cancers, breast cancers contain significantly elevated polyamines. Further, *ODC* was shown to be up-regulated in breast cancer, and its activity and expression was linked to decreased recurrence-free and overall survival (5). However, these earlier studies predated identification of TNBCs as a histological subtype, leaving open the question of whether polyamine metabolism is dysregulated in TNBC and, if so, whether it can be exploited for therapeutic benefit.

To begin, the authors performed a focused metabolomics study, treating MDA-MB-468 and SUM-159PT TNBC cells with either Dox or CsP and examining the impact on arginine-related metabolites. This revealed that whereas ornithine was

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[2] The abbreviations used are: TNBC, triple-negative breast cancer; PD-L1, programmed death-ligand 1; Dox, doxorubicin; CsP, cisplatin; ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine.

the most up-regulated metabolite following chemotherapy, putrescine and spermidine were significantly down-regulated. This pointed them toward the interesting possibility that Dox and CsP, while disparate in mechanism, shared the ability to inhibit ODC, the rate-limiting enzyme catalyzing the conversion of ornithine into putrescine. In strong support of this, the authors found that ODC protein and activity were decreased by both drugs, in a manner dependent upon antizyme (*OAZ1*), a well-characterized negative regulator of ODC. The authors then asked whether direct inhibition of ODC using a highly specific irreversible inhibitor, α-difluoromethylornithine (DFMO) (10), could sensitize TNBC cells to Dox. Indeed, DFMO sensitized five different TNBC cell lines to Dox, whereas non-TNBC cancer cell lines did not display this consistency. Furthermore, the viability of TNBC cells but not non-TNBC cells was consistently decreased by DFMO treatment alone. To understand why TNBC is more vulnerable to ODC inhibition, the authors turned toward the Molecular Taxonomy of Breast Cancer International Consortium and the Cancer Genome Atlas publicly available data sets. Here, *ODC* emerged as one of the *top five* most significantly enriched transcripts in TNBC, at least in part due to Myc-driven transcription (as revealed by a significant correlation between *Myc* gene amplification and *ODC1* transcript levels). Interestingly, the authors found that copy number and transcript levels for members of the antizyme family, which inhibit ODC, were decreased, whereas copy number for the antizyme inhibitor gene, which supports ODC expression by inhibiting antizyme, was increased in TNBC.

This exciting work by Geck *et al.* provides further impetus to pursue the polyamine pathway in TNBC, particularly with respect to its role in chemoresistance, the most significant unmet clinical need in TNBC. The next steps should include examining whether polyamine pathway inhibition sensitizes TNBC cells to various clinically relevant combination regimens (available at RRID:SCR_012959), approximating as much as possible what would be delivered to patients. *In vivo* studies are an essential next step, again approximating as closely as possible clinically relevant combination regimens. Another question that arises is, "What is the best way to target the polyamine pathway?"—especially given that inhibition of polyamine synthesis provokes a rapid compensatory response, reestablishing the intracellular polyamine pool through increased polyamine transport into cells. This ability of tumor cells to override ODC inhibition may explain why DFMO has not enjoyed more clinical success (although it is noted that clinical trials are ongoing in brain cancer (7) (RRID:SCR_002309). Inhibition of the second rate-limiting step in polyamine synthesis, that is catalyzed by SAM decarboxylase (*AMDI*), has likewise met with little clinical success. This has spurred the development of polyamine "analogs" that act as mimics, depleting the intracellular pool through feedback inhibition of endogenous synthesis and stimulation of catabolism. An array of next-generation analogs are under investigation and may provide a superior means to target the polyamine pathway in TNBC. Finally, a strategy termed "polyamine-blocking therapy" (7) in which DFMO is combined with an inhibitor of polyamine transport holds promise. Several studies in immune-competent mouse models suggest that this approach decreases tumor growth while increasing anti-tumor immunity. In summary, this study reveals new possibilities for addressing chemoresistance in TNBC. Exploiting creative innovations in targeting the polyamine pathway will be important for realizing the full promise of these findings.

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