By Melania Tesio

Stemness or Bioenergetics, that is the Question

A new study demonstrates that, in acute myeloid leukaemia, chemotherapy resistance is not linked to stem cell properties but to mitochondrial bioenergetics.

Back in the early 90s, pioneer investigations by Tsvee Lapidot and John Dick provided the first concept of leukemias being organized in a hierarchical fashion. As in physiological hematopoiesis, acute myeloid leukemia (AML) was shown to harbor a rare, self-renewing leukemic stem cell (LSC) population able to initiate disease development and to differentiate into non-self-renewing bulk cells when serially transplanted into immune-deficient mice. Whereas more sensitive xenograft assays refined some aspects of this initial concept over time (ie, a semihierarchical organization rather than a hierarchical one), numerous studies unequivocally identified LSCs and characterized their biologic properties. One of these is the quiescent status, which is believed to render LSCs resistant to cell cycle targeting drugs commonly used in clinical settings, such as cytarabine (AraC).^{1,2} In a recent *Cancer Discovery* issue, Farge et al³ challenged this dogma, demonstrating that the hallmark of chemotherapy resistance is not stemness but rather cellular bioenergetics.

By performing extensive analysis on xenografts obtained from 25 primary AML patient samples, the authors showed that AraC-resistant blasts are neither enriched in LSC frequencies nor in quiescent cells. Consistent with this, cell cycle-related genes were not enriched in the transcriptome of AraC-resistant cells, which instead showed increased expression of transcripts associated with reactive oxygen species (ROS) responses.

Although ROS can be generated by several enzymes in different cellular compartments, the vast majority of them originate in the mitochondria as a consequence of oxidative phosphorylation (OXPHOS). During this process, the progressive oxidation of reducing equivalents generates a transmembrane electrical potential, which in turn drives adenosine triphosphate (ATP) synthesis. Hence, when the authors examined the mitochondrial bioenergetics of AraC-resistant AML cells, they observed increased mitochondrial mass and membrane potential, thus suggesting that AraC resistance is linked to high OXPHOS. To further explore this concept, Farge et al administered AraC to mice xenografted with human AML cell lines differing in their OXPHOS levels. Whereas low OXPHOS AML cells underwent apoptosis following AraC delivery, high OXPHOS cells showed increased treatment resistance *in vivo*. In keeping with this, the pharmacological downregulation of OXPHOS levels enhanced AraC-induced cytotoxic effects both *in vitro* and *in vivo*.

Mitochondrial OXPHOS is fuelled by several different substrates such as glucose, amino acids and fatty acids. AraC-resistant cells markedly upregulated the fatty acid transporter CD36 on their cell surface and, at least *in vitro*, the pharmacological inhibition of fatty acid oxidation (FAO) potentiated AraC cytotoxicity, thus indicating that FAO is most likely to contribute to the high OXPHOS levels observed in AraC-resistant cells. Although these data require further confirmation through *in vivo* experiments, they provide evidence in favor of an important role of the CD36-FAO-OXPHOS axis in therapy resistance. Of note, elevated CD36 expression correlated with a poor clinical outcome.

As evidenced by extensive transcriptomic analysis, chemoresistant OXPHOS high cells preexist treatment: a high OXPHOS signature was observed already at diagnosis in patients whose *in vivo* xenografts showed a low AraC response. High OXPHOS cells not only survived and escaped treatment but they were also counter-selected and amplified by the selective pressure of chemotherapy, as demonstrated by time-course experiments. Hence, in contrast to the cancer stem cell model, whereby relapse is driven by distinct leukemic stem cell populations already present at diagnosis,⁴ in Farge's model, relapse results from preexisting chemoresistant OXPHOS high cells

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Figure 1. In the stem cells model (left), relapse is driven by preexisting leukemia stem cells. These cells consist of 2 distinct populations differing in their immunophenotype but sharing stem properties.⁴ Persisting after therapy, these cells sustain leukemia regrowth owing to their self-renewal capacity. In the bioenergetic model (right), relapse originates from OXPHOS high AML cells. These cells preexist therapy, persist and/or expand following chemotherapy, owing to a metabolic shift of OXPHOS low cells toward an OXPHOS high status.³ The proportion of OXPHOS low and OXPHOS high cells at diagnosis discriminates between high and low AraC responders, respectively. AML = acute myeloid leukemia; AraC = cytarabine; OXPHOS = oxidative phosphorylation.

or/and a metabolic adaptation phenomenon, enabling OXPHOS low AML blasts to acquire an OXPHOS high status in response to therapy (Fig. 1).^{3,4}

As genetic selection seems not to be responsible for this process, how do AML cells adapt their bioenergetics upon AraC therapy? One possible answer to this question involves bone marrow adipocytes. This cell type, expanded in the bone marrow following AraC delivery, has indeed been shown to mediate chemoresistance in different hematological malignancies. An alternative or additional mechanism might rely on a functional transfer of mitochondria from bone marrow stromal cells to AML cells, a process recently shown to elevate ATP production in AML cells and to be enhanced by certain chemotherapeutic drugs.⁵ Cell-autonomous mechanisms could also intervene in this scenario. AraC might, for instance, affect the mitochondrial dynamics of AML cells, altering the balance between fusion (joining of 2 mitochondria into 1) and fission (division of 1 organelle into 2), 2 processes that strongly impact OXPHOS activity.

More work will be necessary to fully understand these interesting aspects and to clarify the discrepancies with previous models of relapse and clonal evolution. Although challenging, metabolic analysis of paired diagnosis and relapse samples might

provide insights into these issues. Similarly, it will be important to establish whether the metabolic adaptation of AML cells is exclusively induced by AraC treatment or instead a phenomenon generally occurring in response to other clinically relevant drugs and therapeutic regimens. Addressing these controversies will be essential to refine relapse predictors and develop novel therapeutic strategies. As in Hegelian dialectic, a thesis necessarily needs a contradicting antithesis to advance cognition.

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