

AUTHOR'S VIEW

More challenges ahead—metabolic heterogeneity of pancreatic cancer stem cells

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ABSTRACT

Pancreatic cancer stem cells (CSCs) display a distinct metabolic phenotype based on their strong dependence on mitochondrial oxidative phosphorylation (OXPHOS) and limited metabolic plasticity. While suppression of MYC upstream of PGC-1 α was the key determinant of this phenotype, we also identified a subset of CSCs with reduced mitochondrial content that showed resistance to mitochondrial targeting, but could be sensitized by inhibition of MYC.

Abbreviations: BET, bromodomain and extra-terminal motif; CSC, cancer stem cell; OXPHOS, oxidative phosphorylation; PDAC, pancreatic ductal adenocarcinoma.

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As demonstrated for some hematologic malignancies and various solid tumors, pancreatic ductal adenocarcinoma (PDAC) is also composed of a heterogeneous population of hierarchically organized cancer cells. Specifically, cancer stem cells (CSCs) represent a functionally distinct subpopulation with exclusive long-term tumorigenicity that also give rise to metastasis and tumor relapse following chemotherapy.^{1,2} CSCs share properties with normal stem cells, such as unlimited self-renewal capacity, differentiation into progenies that form the bulk of the tumor, and strong resistance to xenobiotics.²

We have recently demonstrated that *in vivo* treatment with the antidiabetic drug metformin, which blocks mitochondrial function via inhibition of complex I, led to initial tumor regression and extended the survival of preclinical mouse models.³ The observed response was the result of cell cycle arrest in more differentiated cancer cells (non-CSCs), and, even more importantly, induction of apoptotic death in CSCs. This differential response to mitochondrial inhibition of pancreatic cancer cells strongly suggested a distinct metabolic phenotype for CSCs.

Our subsequent mechanistic studies revealed that, indeed, a large, but variable, fraction of the pancreatic CSCs harbored in each individual tumor was particularly sensitive to mitochondrial inhibition due to their strong dependence on oxidative phosphorylation (OXPHOS). As such, treatment with inhibitors of mitochondrial function—for example the mitochondrial reactive oxygen species (ROS) inducer menadione, the ATP synthase inhibitor resveratrol, or the complex I inhibitor rotenone—preferentially induced mitochondrial ROS production and apoptosis, drastically diminishing the number of CSCs both *in vitro* and *in vivo*. We further demonstrated that mitochondrial targeting was particularly lethal for CSCs because of their limited metabolic plasticity; surprisingly, CSCs showed a low rate of glycolysis activation following inhibition of

mitochondrial respiration, which eventually translated into ATP depletion and energy crisis in the cells.⁴

Importantly, however, tumors ultimately relapsed under metformin treatment due to the outgrowth of resistant CSC subclones.^{3,4} We determined that CSCs isolated from these arising metformin-resistant tumors were distinct from the main populations of CSCs found in each tumor based on their intermediate metabolic phenotype and enhanced metabolic plasticity, thus rendering them resistant to mitochondrial inhibition. These data were further corroborated by studying the clonal composition of vehicle-treated *versus* metformin-treated tumors using a barcode tracking library. We found a significant enrichment of certain barcodes in metformin-treated tumors, which in some cases represented up to 15% of the tumor, demonstrating that only a subset of CSCs was, or became, resistant to metformin and consistent with a strong metabolic heterogeneity of treatment-naïve CSCs.

As predicted by our data, results from recent clinical trials using metformin in combination with standard therapies for locally advanced and metastatic PDAC revealed no sustained treatment effects,⁵ which can be attributed to the rapid outgrowth of resistant clones. Thus, unraveling the mechanisms driving intratumoral metabolic heterogeneity was of utmost importance for further clinical development of this concept. Among the genes that were differentially expressed in metformin-sensitive *versus* resistant CSCs, we identified the mitochondrial biogenesis transcription factor peroxisome proliferator-activated receptor gamma coactivator 1- α (PPARGC1A, best known as PGC-1 α), which is negatively controlled by the V-Myc avian myelocytomatosis viral oncogene homolog (best known as c-MYC), as the major determinant for the enhanced mitochondrial respiration in CSCs. Importantly, mitochondrial mass as determined by Mitotracker-Deep Red (Mito) staining served as a surrogate marker for PGC-1 α

expression, which, in combination with the CSC surface marker prominin-1 (also known as CD133), allowed us to discriminate 2 subpopulations of CSCs (Fig. 1A). On the one hand, CD133⁺/Mito^{high} cells were strongly enriched for CSCs (as demonstrated by an increased expression of pluripotency-associated genes, self-renewal, and CSC frequency) and responded well to metformin in terms of induction of apoptosis. On the other hand, CD133⁺/Mito^{low} cells showed reduced mitochondrial respiration and thus resistance to metformin, albeit at the expense of reduced stemness. The metabolic phenotype and resistance to metformin displayed by the latter population of cells was reminiscent of that found in CSCs isolated from metformin-resistant tumors.

Although none of these findings actually disproved acquired resistance as a contributing factor, our data strongly support the pre-existence of metabolic heterogeneity within the CSC compartment in treatment-naïve pancreatic cancer. These CSCs were able to expand under metformin treatment and drove tumor progression/relapse. Interestingly, such CD133⁺/Mito⁺ cells with high mitochondrial mass have also been described for pancreatic tumors of murine origin,⁶ indicating that such heterogeneity may be rather universal and not restricted to human pancreatic cancer. Indeed, our data are in

line with several recent studies reporting diverse subsets of cancer cells with different metabolic requirements that reside within various tumor entities.^{7,8} The co-existence of CSC subsets differing in their oxygen metabolism has also been suggested for established head and neck squamous cell carcinoma cell lines with hypoxia inducible factor 1 α (HIF-1 α) as a major determinant.⁹ Moreover, metabolically distinct subsets of CSCs have been found in relation to an epithelial-mesenchymal transition (EMT) phenotype.¹⁰ However, this matter is further complicated by the fact that functionally distinct CSCs subpopulations may interact with the tumor microenvironment through secreted and excreted metabolic products. This could constitute yet another level of complexity in tumor metabolism in which the CSC niche may substantially contribute to the regional/clonal metabolic phenotype.

In conclusion, only the simultaneous targeting of all the different subpopulations of cancer cells present in treatment-naïve tumors should avoid the later outgrowth of resistant clones and subsequent relapse of the disease. This will require the development of multimodal therapies against the different cellular components responsible for both functional and metabolic intratumoral heterogeneity in order to achieve durable responses. Intriguingly, in our study the addition of the BET (bromodomain and extra-terminal motif) inhibitor JQ1 was capable of preventing and overcoming resistance via downregulation of MYC (Fig. 1B). This forced all CSCs into the utilization of OXPHOS and rendered them sensitive to mitochondrial targeting. Thus, our findings indicate that the combination of OXPHOS and c-MYC inhibition represents a novel multimodal approach for more effectively targeting the distinct, but heterogeneous metabolic features of pancreatic CSCs.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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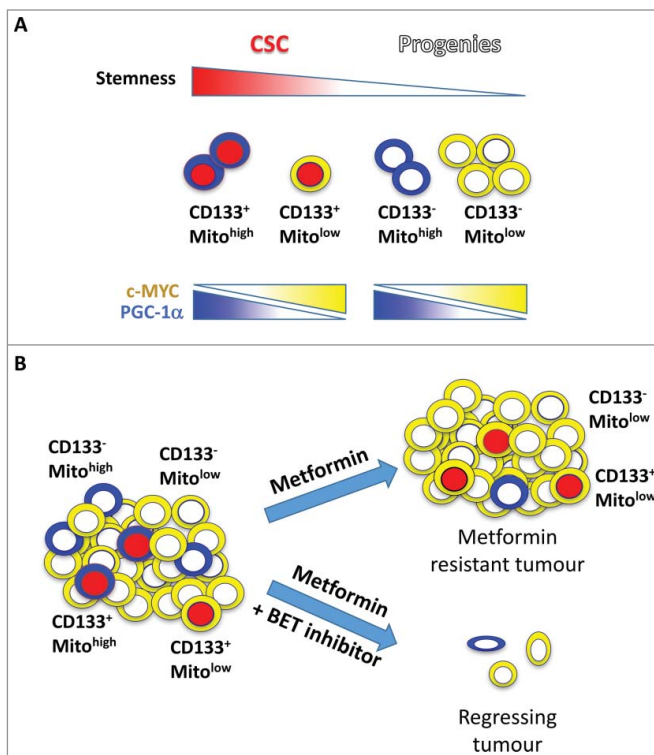


Figure 1. Heterogeneity in pancreatic cancer cells. (A) Schematic representation of cancer cell subsets found in pancreatic tumors, classified by stemness (cancer stem cells (CSC), identified by CD133⁺ expression; progenies as CD133⁻) and mitochondrial mass (Mito), dependent on MYC/PGC-1 α expression (Mito^{high}, cells with high mitochondrial mass, expressing PGC-1 α associated with an OXPHOS phenotype; and Mito^{low}, cells showing low mitochondrial content, with MYC driving a Warburg-like metabolic state). (B) Effect of single versus multimodal treatment with metformin: single treatment with metformin only eliminates CD133⁺/Mito^{high} CSCs, leading to rapid tumor relapse and resistance to metformin; multimodal treatment with BET (bromodomain and extra-terminal motif) inhibitors prevents/overcomes resistance to metformin, targeting all cellular subsets that compose the tumor.

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