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INSIGHTS

Pancreatic cancer *SLUG*ged

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In this issue of *JEM*, Recouvreux et al. (https://doi.org/10.1084/jem.20200388) describe the role of nutrient sensing in the induction of epithelial–mesenchymal transition. Glutamine-deficient pancreatic cancer cells up-regulate classic EMT regulator Slug, providing a link between nutrient stress and metastasis.

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy and the fourth leading cause of cancer-related deaths worldwide. Constituting ∼90% of all histological types of pancreatic cancers, PDAC is typically a fatal diagnosis. Mortality in many of these cases can be attributed to various factors including the lack of early detection methods, a paucity of effective therapies, and common early metastasis. Thus, significant research is dedicated to these topics in order to improve patient outcomes. With the recent development of animal and organoid models, PDAC also serves as a proto-typical malignancy in which general concepts of fundamental cancer biology can be readily explored. In this issue of *JEM*, Recouvreux et al. (2020) explore glutamine dependency in PDAC, and thereby uncover a link to cell differentiation states and epithelial–mesenchymal transition (EMT), providing new mechanistic insights into the process of metastasis.

The poor outcomes of PDAC patients have also been ascribed to the hypoxic and nutrient-limited environment observed in PDAC tumors. Arising from the acinar and ductal cells in the exocrine pancreas, PDAC tumors are notorious for having a deficient vascular supply with poor perfusion (Olive et al., 2009). Chronic nutrient stress and hypoxia can provide selective pressure for the outgrowth of cancer cells resistant to these conditions, which may contribute to the previously recognized capacity of PDAC to metastasize early (Rhim et al., 2012).

The study of the tumor microenvironment in PDAC has demonstrated the importance of glutamine to tumor cells. Access to glutamine is required for cell proliferation, and in the case of PDAC, for survival. Glutamine can be used as a source of both carbon and nitrogen, contributing to a wide array of cellular processes, including amino acid synthesis, lipid synthesis, and energy production via the TCA cycle. In cancer cells, however, the contribution of glutamine to the TCA cycle via anaplerotic pathways is notably increased (Kamphorst et al., 2015).

In the case of PDAC, it has been shown that macropinocytosis, or the ability of cells to engulf extracellular stromal constituents, is a major contributor to intracellular glutamine. Prior work by this team found that, through oncogenic activation of *KRAS*, PDAC cells activate macropinocytosis to generate nutrients for use as a carbon source for energy production and biomass (Commisso et al., 2013). It was later determined that free glutamine is surprisingly only poorly taken up from the PDAC microenvironment, confirming that host-derived albumin and other extracellular matrix proteins are the main sources of glutamine within the tumor (Sullivan et al., 2019).

Rather than converting glutamine sequentially to glutamate and then α-ketoglutarate, PDAC cells have been found to preferentially metabolize glutamine by cytosolic transaminases into malate and pyruvate. This process increases the NADPH pool within the cell, increasing the cellular capacity to synthesize fatty acids, reduce glutathione, and protect against ROS stress to thereby influence cell proliferation and survival (Son et al., 2013). This finding exposes a unique glutamine dependency in PDAC cells, demonstrating their reliance on glutamine for proliferation, biomass synthesis, and redox regulation.

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In the setting of reduced glutamine, the authors found that PDAC cells activate the integrated stress response pathway. This pathway is involved in determining whether neoplastic cells become quiescent to preserve resources, remain proliferative while remaining spatially localized, or activate biological programs that promote migration to a new environment (Senft et al., 2016). A common method of cell migration involves the up-regulation of specific transcription factors that increase cell motility and tissue invasion, promoting the transition from an epithelial to a mesenchymal

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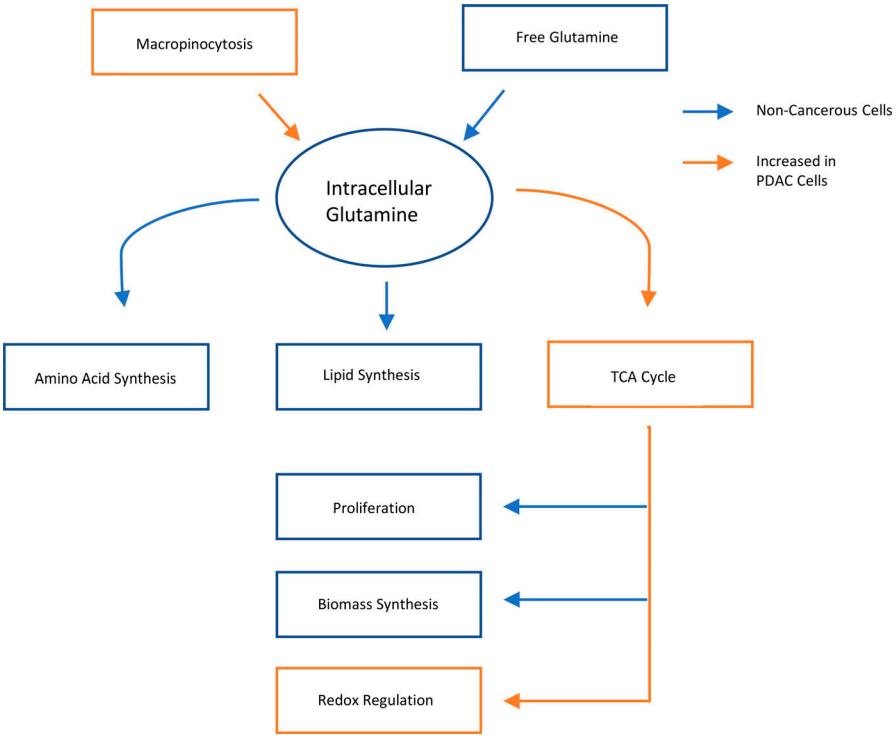

Simplified description of glutamine usage in noncancerous versus PDAC cells.

cell differentiation state, codified as EMT. EMT is a normal cellular process that is required for embryonic development, and many aspects of physiological EMT are appropriated toward malignant EMT. For example, up-regulation of transcription factors such as *Snail* and *Slug* leads to increased expression of mesenchymal genes (fibronectin, N-cadherin, etc.) and decreased expression of epithelial genes (E-cadherin, ZO1, etc.; Lu and Kang, 2019). Cell-based in vitro systems have shown that enforced expression of these transcription factors are sufficient for inducing EMT, providing a more mobile cellular state that resemble fibroblasts.

Snail expression has previously been shown to stall the cell cycle and provide temporary resistance to pro-apoptotic signals and cell death through the loss of survival factors. Thus, while EMT allows for specialized cells to migrate and proliferate, in other contexts, EMT can lead to a reduced sensitivity to important cell death signals. As a member of the *Snail* family, *Slug* is a transcriptional repressor of E-cadherin and multiple proliferation pathway genes (Vega et al., 2004). Through inactivation of these pathways, Slug decreases metabolic demand, an important function in the context of nutrient stress.

In recent years, there has been apparent controversy surrounding the role of EMT in PDAC. Although many prior studies suggested that EMT is critical for PDAC metastasis, it was reported that two key EMT transcription factors, *Snail* and *Twist*, were not required to promote PDAC metastasis and invasion (Zheng et al., 2015). However, subsequent studies showed that another regulator of EMT, *Zeb1*, was required to promote PDAC metastasis, suggesting that EMT drivers are not equal in PDAC (Krebs et al., 2017). Thus, further research is still needed to fully determine role and mechanism of EMT in PDAC.

In this report, Recouvreux et al. (2020) demonstrate a link between nutrient stress and EMT activation in PDAC cells, showing that glutamine deprivation plays a major part. Specifically, they report that glutamine limitation up-regulates the expression of EMT transcription factor *Slug* via MAPK signaling and ATF4 activation, a well-known stress response pathway that gets activated in response to nutrient starvation. The mechanistic basis for the activation of these pathways by glutamine deprivation remains to be clarified. Nonetheless, given *Slug*'s canonical role in EMT, they further found that glutamine limitation enhances the migration and invasion capacity of PDAC cells, potentially providing a link to its metastatic abilities. They correlate their laboratory work with prior clinical data that had previously identified an inverse relationship between *Slug* expression and outcomes in human PDAC.

The connection between glutamine deficiency and EMT in PDAC is important and timely. While PDAC is unique regarding its unusual microenvironment with impaired perfusion, recent careful studies using preoperative pimonidazole (Dhani et al., 2015) and noninvasive imaging with the positron emitter 18F-fluoroazomyin arabinoside (Metran-Nascente et al., 2016) did not detect substantial hypoxia in the majority of PDAC specimens and suggest that hypoxia would not be the major driver of the metastatic propensity of PDAC. Therefore, glutamine deficiency is an attractive alternative metabolic deficiency to trigger EMT and metastasis in PDAC. Additionally, the recent finding that increased oncogenic *KRAS* dosage promotes the more basal subtype of PDAC, which correlates with invasion and metastasis and hyperactivates the MAPK effector pathway (Miyabayashi et al., 2020), may be the setting in which certain PDAC cells are more prone to respond to sub-optimal glutamine levels and activate *Slug* expression. It will also be relevant to determine whether these observations extend to additional tumor types, and if so, whether intratumoral glutamine levels per se are predictive of metastatic propensity. Finally, the field should search for additional biochemical and biophysical (Laklai et al., 2016) microenvironmental factors that may separately induce EMT and promote tumor progression.

This new observation linking glutamine deficiency in PDAC to increased metastatic potential may also represent a therapeutic opportunity. For example, glutaminase inhibitors prevent the deamination of glutamine to glutamate before its incorporation into anaplerotic pathways such as the Krebs cycle (Koch et al., 2020); in principle, such inhibitors would raise intracellular and intratumoral glutamine levels to avoid triggering the MAPK and AT4-dependent *Slug* up-regulation. Not only could this decrease metastatic spread, but targeting *Slug* could also reduce the tumor's resistance to pro-apoptotic signals and cell death to engender improved responses to therapies. In closing, Recouvreux et al. (2020) have made

a noteworthy contribution to the field of PDAC research, linking the fields of cancer metabolism and disease pathogenesis.

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