Mathematical Models of Metabolism

Metabolism is essential for life, and its alteration is implicated in multiple human diseases. The transformation from a normal cell to a cancerous cell requires metabolic changes to fuel the high metabolic demands of the cancer cell, including but not limited to cell proliferation and cell migration. Our group investigates cancer metabolism from an evolutionary point of view. We hypothesise that given specific microenvironmental conditions and metabolic constraints, there is an optimal mode of cell metabolism to achieve a metabolic objective. This metabolic mode will offer an evolutionary advantage and therefore will be selected for during the time course of cancer development. First, we aim to uncover the metabolic objectives and metabolic constraints upon which natural selection is acting. Second, we aim to determine which known (and yet to be discovered) molecular alterations are driving the deterministic or stochastic occurrence of the optimal metabolic modes.

Increased formate overflow is a hallmark of cancer

In his 1956 landmark paper, Otto Warburg hypothesised that cancer is caused by mitochondrial defects that result in increased rates of glycolysis with lactate overflow. Today, increased glycolysis is an established hallmark of cancer metabolism and forms the scientific basis for Positron Emission Tomography (PET) scans. In contrast, the Warburg hypothesis that cancer harbours defective mitochondria has remained controversial. Recent evidence indicates that some tumours have rates of glucose oxidation comparable to those observed in normal tissues, challenging the assumption that cancer cells are characterised by defective mitochondrial metabolism. A pathway that relies on functional mitochondria is the oxidation of the third carbon of serine to formate. Formate produced in the mitochondrial one-carbon metabolism to formate in normal tissues is released into the cytosol, where it supplies the one-carbon demand for nucleotide synthesis (Fig. 1). Formate can also be recycled back to serine by cytosolic formate dehydrogenase and thereby can be used as an alternative one-carbon unit.

In 2011, while working at the Rutgers Cancer Institute of New Jersey, I predicted that serine catabolism, and subsequent formate production, should occur at rates exceeding the one-carbon demand of biosynthesis (Vazquez et al. Cell 145, 2011; 6: e25888). The excess formate would be released from cells, a process referred to as formate overflow. In 2016, our laboratory experimentally verified that genetic knockdown of MTHFD1L, the enzyme responsible for formate production in the mitochondrial one-carbon metabolism, causes the elevated plasma formate levels. Moreover, we have recently observed that inhibition of formate production by genetic knockdown reduces invasion and that this phenotype can be rescued by exogenous formate (Fig. 2). We will continue these studies to decipher the mechanism of the formate-dependent induction of invasion. We conclude that some cancers are characterised by significant oxidative metabolism, and we identify formate overflow as the hallmark of such oxidative cancer types. Furthermore, we propose cell invasion as a key selective advantage of formate overflow.

In parallel, we have conducted phenotypic studies to determine the potential selective advantage of formate overflow. Using multiple cancer cell lines, we have shown that genetic knockdown of MTHFD1L, the enzyme responsible for formate production in the mitochondrial one-carbon metabolism, does not result in any significant change in cell proliferation. Yet we have recently observed that inhibition of formate production by genetic knockdown reduces invasion and this phenotype can be rescued by exogenous formate (Fig. 2). We will continue these studies to decipher the mechanism of the formate-dependent induction of invasion. We conclude that some cancers are characterised by significant oxidative metabolism, and we identify formate overflow as the hallmark of such oxidative cancer types. Furthermore, we propose cell invasion as a key selective advantage of formate overflow.

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**Figure 1** Increased serum formate levels in genetically engineered mouse models (GEMMs). (a–d) Serum formate in wild-type (WT) pre-neoplastic (P) and neoplastic (N) mice from different GEMMs for (a) intestinal cancer; (b) mammary carcinoma; (c) lymphoma, and (d) sarcoma. (e) Intestinal adenomas of APC Min/+ mice. All the tumor-specific high-serine catabolism causes the elevated plasma formate levels.

**Figure 2** Formate overflow promotes cancer cell invasion in glioblastoma. (a) Cartoon illustrating the experimental setup to analyse cancer cell invasion using coated Boyden chambers. (b–e) Addition of extracellular sodium formate increases invasiveness in a concentration-dependent manner in (b) U87. (c) LN229 and (d) NCH601 cells. (f) Reduced invasiveness by MTHFD1L knockdown can be rescued with extracellular formate in (d) LN229 and (e) NCH601 cells.