EDITORIAL

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Two minutes and the two µM oxaloacetatemakes the phenotypic differences betweenproliferative and differentiation and the metastatic cells escape acidic microenvironment in search of a new life

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Abstract:

Present model of intermediary metabolism has its origins in the first quarter of 20th century, developed by Meyerhof, who worked on muscles and Warburg on the cancers. Johnson and Krebs proposed the citric acid cycle, popularly known as the TCA cycle or Krebs cycle, when they observed that addition of small amounts of pyruvate increased the succinate levels in pigeon breast muscle extracts. They proposed the cyclic nature of the citric acid cycle based on two contemporary reports. Szent Gyorgyi reported that dicarboxylate oxidations supply hydrogens to oxygen to reduce it to water during the respiration. Martius group proposed that citric acid oxidation results in the production of the α-ketoglutarate (α-KG), but citric acid does not disappear from the reaction mixture. Besides, they proposed that the equilibrium of the first reaction, catalyzed by the aconitase favors the formation of 90% of citrate, and further proposed that isocitrate dehydrogenase (IDH) produces an intermediate oxalosuccinic acid, which is spontaneously decarboxylated to α-KG. Johnson in a later study confirmed that the equilibrium of aconitase reaction favors >80% of citrate formation. In extensive studies on citrate production, between 1945 -1960, Ochoa and his collaborators first proposed that citrate production involves the reductive carboxylation of the oxalosuccinate to citrate, and when the reaction is carried out in the presence of the glucose-6phosphate dehydrogenase (G6PDH) of the oxidative pentose phosphate pathway (oxPPP) and aconitase, Mg2+, and with NADPH as the cofactor, favors 90% citrate production.

When Lipmann reported that acetylation reactions require the activation of the coenzyme A (-CoA), Ochoa and Stern reported the presence of the enzyme citrate synthase (CS), but reported that the CS reaction is active only for two minutes and that two, when the concentration of the oxaloacetate is <2µM and if concentration exceeds 2µM, oxaloacetate inhibits the reaction. Krebs ignored the reductive carboxylation mediated citrate synthesis and adopted the CS reaction as the mechanism of citrate production in support of his TCA cycle in his Nobel lecture (1953). Since then, pyruvate remained the gatekeeper of the glucose metabolism in the mitochondria, and mitochondria as churning machines to degrade the nutrients to CO2 to produce ATP. Citrate production in the mitochondria remains the symbol of mitochondrial respiration and as the fulcrum between the carbohydrate and fatty acid production. Recent studies on Glioblastomas recognize the role of three isoforms of IDH, IDH 1, 2 & 3, and two mutants of IDH1 & 2 in citrate production and carcinogenesis. A series of papers in 2011 reported by Wise et al. (PMID: 22106302), Metallo et al. (PMID: 22101433) Mullen et al. (PMID: 22101431) suggested that reductive carboxylation of the α-KG is the principal contributor of citrate synthesis; all these authors use a rider " hypoxic environment" or "damaged mitochondria" while reporting their results. Interestingly, one subsequent paper by Mullen et al. (2014, PMID: 24857658) reported that reductive carboxylation needs the support of oxidation of α-KG to Succinate. Jiang et al 2016 reported that reductive carboxylation by IDH1 supports the redox homeostasis in anchorage independent cells (PMID: 27049945). While Mullen et al.'s 2014 paper gives the reason for Johnson and Krebs's observation that addition of pyruvate increased the succinate levels. Succinate inhibits the NADH oxidation in respiratory chain and conserves hydrogen by transhydrogenation to NADPH for biosynthesis.

The discovery of two mitochondrial carrier proteins, the MPC1 and MPC2, and the demonstration that lactate entry into cells through monocarboxylate transporter 1 (MCT1) activates the cell surface receptor HCAR1 and inhibits the cyclic AMP signalling and activates HIF2 dependent glutamine and leucine uptake. Lactate activates the mitochondrial glutamine and BCAA metabolism. The primary problem of the research reports over the past 100 years is that aerobic glycolysis and TCA cycle are the Gold standards of intermediary metabolism, and anything deviating from these reports is either an error or due to mutation. As the organizers belong to the nursing fraternity, they should have known that prenatal and post-natal life of an infant is a growth period, and a baby fed on mother's breast milk will grow healthy to adulthood. Growth takes place under the influence of hypoxic inducible factors, not necessarily at low oxygen levels, but on the ratio of glutamate to α-KG, which is maintained by the De Ritis ratio of aminotransferases (GPT1/2, GOT1/2, BCAT1/2) and the glutamate dehydrogenase GLUD during proliferation and differentiation.

The extracellular pH (pHe) of tumor tissues is often acidic [1], and acidic metabolites, e.g. lactic acid caused by anaerobic glycolysis in hypoxia, seem to be the main cause. Accumulating evidence shows that an acidic microenvironment is a regulator of cellular phenotype. Whereas Na+-HCO3- co-transporter and Cl-/HCO3- exchanger contribute a fall in intracellular pH, the Na+/H+ exchanger (NHE) [2], the H+-lactate co-transporter, monocarboxylate transporters

Acidic extracellular pH is a major feature of tumor tissue, extracellular acidification being primarily considered to be due to lactate secretion from anaerobic glycolysis. Clinicopathological evidence shows that transporters and pumps contribute to H+ secretion, such as the Na+/H+ exchanger, the H+-lactate co-transporter, monocarboxylate transporters, and the proton pump (H+-ATPase); these may also be associated with tumor metastasis.

An acidic extracellular pH not only activates secreted lysosomal enzymes that have an optimal pH in the acidic range, but induces the expression of certain genes of pro-metastatic factors through an intracellular signaling cascade that is different from hypoxia. In addition to lactate, CO2 from the pentose phosphate pathway is an alternative source of acidity, showing that hypoxia and extracellular acidity are, while being independent from each other, deeply associated with the cellular microenvironment.

The report of Jiang et al. on anchorage independent cells reminds the one and half century old observation of Claude Bernard that tissue turn over maintains homeostasis, and the recent reports the TGF-β and cytokine induced cell transformation produces anchorage independent cells. TGF-β inhibits the cell surface antigen 4F2HC/ CD98HC in the anchorage independent mesenchymal cells, which prevents cell surface glutamine and leucine uptake. Anchorage independent mesenchymal depend on the non/ conditionally essential amino acids liberated by the hydrolysis of collagen in the microenvironment. TCA cycle derailed the research on the de novo cholesterol and fatty acids biosynthesis. The initial work of Konrad Bloch on the leucine metabolites (dimethylated branched-chain carboxylic acids) as the key mediators of the cholesterol biosynthesis, was derailed by World War II and migration of scientists from Europe. The conventional models of metabolism fail to explain as to how cholesterol levels are elevated, and the de novo fatty acid synthesis is inhibited under mitochondrial dysfunction.