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A 3D view of Barth syndrome mitochondria

For several decades, electron microscopy has provided pivotal information to pathologists on a large array of disease states that are associated with characteristic abnormalities of intracellular structures. However, an inherent limitation of conventional transmission electron microscopy is that it shows a more or less random 2D projection image of the object in question. In this issue, **Acehan *et al***¹ (p. 40) used electron microscope tomography to study the 3D structure of mitochondria in patients with Barth Syndrome. Barth Syndrome is an X-linked multisystemic disease caused by a defect in the metabolism of the mitochondrial phospholipid cardiolipin.^{2,3} Abnormal mitochondria have been documented in Barth patients, but the exact nature of the alterations in membrane architecture has remained elusive. The authors made use of conventional electron microscopy for statistical analysis of structures and collected tomography data of several representative areas for a detailed comparison. They have confirmed earlier observations about variations of mitochondrial population and size distribution in Barth Syndrome lymphoblasts compared to wild-type cells. Their work also revealed general organization of the cristae within the mitochondria where wild-type cells have an evenly spaced parallel distribution and Barth Syndrome mitochondria have their cristae concentrated close to the periphery of the mitochondrial volume. (In addition, most of the lamellar cristae studied in lymphoblasts showed a slot-like connection to the mitochondrial outer membrane.)

The authors' findings using detailed 3D comparisons of cristae structures are noteworthy. They report a novel feature of Barth Syndrome mitochondrial cristae, wherein some regions the membranes are very close to each other, resulting in minimization or deletion of crucial inter membrane space. Another observation that can be attributed to the 3D nature of their study was the discovery of novel tubular structures that were found only in mitochondria from Barth Syndrome patients. These structures seem to be connected to the rest of the mitochondrial cristae network.

Electron microscope tomography has been evolving rapidly due to advances in hardware and computer programs in the last years. This methodology is sure to gain more popularity in pathology, where 3D models can take away much of the ambiguity of 2D electron micrograph images, and

reveal details otherwise invisible or hard to confirm. This method has also the potential to combine results from other 3D methods and biochemical studies to give a comprehensive understanding of structures and events at the molecular level.

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Imaging lactate: pyruvate ratios in tissues

The most celebrated scientific effort in the eyes of the public is that being given to understanding the macromolecular basis of human disease, namely, derangement in nucleic acids and proteins. An overlooked fact is that it is the small molecules of intermediary metabolism that provide the metabolic energy for sustenance of life, and the building blocks for macromolecules. In the first instance, the most central of all metabolic pathways is glycolysis and oxidative metabolism via the Krebs cycle. Through this catabolic pathway is provided much of the ATP for cellular function. At the crossroads between glycolysis/gluconeogenesis and the Krebs cycle is pyruvate. In the catabolic pathway of glycolysis, pyruvate dehydrogenase consumes pyruvate to generate acetyl:CoA, which enters into the Krebs cycle. In the anabolic pathway of gluconeogenesis, oxaloacetate leaves the Krebs cycle via oxaloacetate decarboxylase to generate pyruvate. As the substrate or product of multiple highly regulated enzymes, pyruvate concentrations within the cell are generally low. A key sidestep in this metabolic crossroads is lactate dehydrogenase, which is a bidirectional enzyme regulated by ambient levels of [NAD⁺]/[NADH + H⁺]. High NADH levels occur in well-oxygenated tissues, and in turn drive the conversion of pyruvate to lactate via lactate dehydrogenase. Low NADH levels reflect anaerobic conditions, and drive the conversion of lactate to pyruvate. Hence, the lactate-to-pyruvate (L/P) ratio in tissues is theoretically a sensitive indicator of the oxidative status of cellular metabolism. In homogeneous tissues such as liver and muscle, measuring the

L/P ratio on tissue sample is a valid analysis, since it can be reasonably assumed that there is relatively uniform metabolic status among the parenchymal cells in the tissue. However, this assumption breaks down in nonuniform tissues, such as malignant solid tumors. As there are many intervening stromal elements, and since the tumor mass may contain residual native parenchymal tissue, a 'homogenized' analysis—whether by spectroscopic or enzymatic means—may be quite futile. In the current issue, **Sattler et al**¹ (p. 84) took advantage of bioluminescent techniques for measuring ATP, glucose and lactate, to develop a quantitative bioluminescent technique for measuring pyruvate. Bioluminescent imaging of lactate and pyruvate in adjacent tissue sections then permitted identification of the L/P ratios in solid human tumors—information which is rarely available. As the redox state of tumor cells might be critical for the efficiency of irradiation and a number of chemotherapeutics, and since lactate and pyruvate are known to have radical scavenger functions, it is likely that a correlation exists between the L/P ratio and radiosensitivity of tumors. As this imaging technique provides both quantitative and structural information, this paper represents an important advance in being able to obtain metabolic maps that reveal tumor heterogeneity. This work is therefore highly significant for advancing research in tumor biology, as well as improving diagnosis and prognosis assessment of cancer in a reproducible, standardized manner.

Reference

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mTOR activation in PTLD

Post-transplant lymphoproliferative disorder (PTLD) is a major complication of both solid organ and allogeneic bone marrow transplantation. Although immunosuppression and reactivation of Epstein–Barr virus (EBV) have been implicated in the development of most of the cases, PTLDs are rather heterogeneous in histopathology, and their pathogenesis is not fully understood. In this issue, **El-Salem et al**¹ (p. 29) demonstrate constitutive activation of the mammalian target of rapamycin

(mTOR) signaling pathway, namely p70S6 kinase (S6K1) and 4E-BP1, in primary, patient-derived tissue samples representing various types of PTLDs. The study shows that the mTOR pathway is activated in the entire spectrum of PTLD subtypes regardless of their EBV genome expression status, thus providing a pathobiological evidence that the mTOR pathway is ubiquitously activated in PTLDs.

mTOR is a protein kinase that controls cell growth by regulating many cellular processes, including protein synthesis and autophagy. Accumulated evidence indicates that mTOR integrates input from multiple upstream pathways, including insulin, growth factors, nutrients, mitogens and energy. Of interest, the mTOR pathway is hyperactive in certain cancers, suggesting mTOR as an attractive target for cancer therapy.² The authors previously demonstrated that EBV-transformed B lymphocytes are sensitive to mTOR inhibitors.³ Taken together with the present study, these indicate that mTOR inhibitors, such as rapamycin and its derivatives, may be effective in treatment and prevention of PTLDs. This is particularly interesting considering the fact that reduction or withdrawal of immunosuppression is the cornerstone of the current treatment of PTLDs, which accompanies the risk of allograft rejection. As mTOR inhibitors have potent immunosuppressive effects, they may potentially act in the setting of PTLD as dual-function drugs by suppressing growth of PTLD cells and, at the same time, preventing immune rejection of the transplanted organ.

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