Research Techniques Made Simple: Profiling Cellular Energy Metabolism

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The analysis of cellular metabolism is attracting increasing interest. Glycolysis and oxidative phosphorylation are intertwined with one another and dozens of other pathways to ultimately produce energy and maintain cellular fitness. However, cellular metabolism is much more than this. Metabolism underlies the proliferation, differentiation, and function of cells as well as the coordination of intercellular communication. Investigating metabolism allows the interpretation of cellular behavior in health and disease. In this article, we aim to demystify the complexity of cellular metabolism and explain the common approaches to study it. Whereas the analysis of cellular metabolism by western blot or flow cytometry might be accessible to most investigators, the functional and comprehensive analyses obtained with a Seahorse Analyzer or mass spectrometer come with monetary and logistical hurdles. We believe that the application of these techniques, together with collaborative efforts between scientists and clinicians, will uncover disease mechanisms and open novel therapeutic avenues for unmet clinical needs in the field of dermatology.

Journal of Investigative Dermatology (2021) **141,** 2767–2774; doi:10.1016/j.jid.2021.09.004

Introduction

Cellular metabolism is fundamental to life. The breakdown of biomolecules results in the generation of metabolic energy in the form of adenosine triphosphate (ATP), which is needed for almost all cellular activities. Moreover, metabolic reactions and the resulting metabolites (hundreds of small molecules) provide the substrates for a cell to synthesize macromolecules such as proteins, carbohydrates, lipids, and nucleic acids. However, pivotal roles of metabolism beyond these well-known functions have recently emerged. The intense cross-talk between metabolic pathways and other cellular signaling cascades, for instance, those involved in immune responses, development, or aging, determines cell behavior and thus pathophysiology.

In principle, cells generate ATP through two major pathways: glycolysis (aerobic or anaerobic) and mitochondrial respiration (i.e., oxidative phosphorylation [OXPHOS]). A key source of energy is the breakdown of glucose, termed glycolysis, in which a chain of enzymatic reactions converts glucose to two units of pyruvate in the cytosol. Under anaerobic conditions, pyruvate is then converted to lactate. However, in aerobic conditions, pyruvate is shuttled into the mitochondrial citric acid cycle (tricarboxylic acid [TCA] cycle), where it gets oxidized, yielding carbon dioxide. This process generates reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$), which are used by the electron transport chain (ETC) for the coordinated movement of electrons across the inner mitochondrial membrane (IMM) converting oxygen to water. The ETC is organized in complexes (complexes I, II, III, and IV). In a simplified view, complexes I, III, and IV transport protons from the mitochondrial matrix into the intermembrane space (between the IMM and the outer mitochondrial membrane [OMM]) in specific invaginations of the IMM called cristae. The mitochondrial ATP synthase (often called complex V) then couples the energy stored in the proton gradient across the IMM to the synthesis of ATP (OXPHOS). Of note, producing ATP through OXPHOS is far more efficient than glycolysis. In addition to glucose, acetyl-coenzyme A derived from the oxidation of fatty acids and carbons from protein-derived amino acids are important sources for fueling the TCA cycle.

One of many examples of how core metabolic pathways are linked to cellular phenotypes and functions is the role of exogenous lipids in cutaneous $CD8^+$ tissue-resident memory T cells. Their survival depends on the uptake of exogenous lipids fueling OXPHOS (Pan et al., 2017). Interestingly, T cells metabolically adapt to the lipid environment at different anatomical sites, by regulating the expression of distinct FABP for fatty acid uptake (Frizzell et al., 2020). In the cutaneous lipid environment, two members of the family, FABP4 and FABP5, play a pivotal role, and their loss is linked to reduced T-cell survival and function (Pan et al., 2017).

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Abbreviations: ATP, adenosine triphosphate; ETC, electron transport chain; IMM, inner mitochondrial membrane; KC, keratinocyte; OCR, oxygen consumption rate; OMM, outer mitochondrial membrane; OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid; TEM, transmission electron microscopy; TMRM, tetramethylrhodamine, methyl ester

SUMMARY POINTS

Advantages

- Western blot is cheap and quick for assessing mitochondrial properties (Jha et al., 2016).
- Imaging allows for single-cell resolution and live-cell imaging (Gökerküçük et al., 2020).
- Transmission electron microscopy (TEM) can zoom in on a single mitochondrial ultrastructure (Vincent et al., 2016).
- Flow cytometry is a quick and easy way to resolve metabolic states at a single-cell level, with the possibility of simultaneously testing multiple cell subsets.
- SCENITH allows one to functionally test the dependency of cells on different metabolic pathways, with the possibility of simultaneously testing multiple cell subsets (Argüello et al., 2020).
- Seahorse allows for real-time functional quantification of cellular energetics on a relatively low number of live cells (van der Windt et al., 2016).
- Metabolomics provides a comprehensive study of cellular metabolites (Jang et al., 2018).

Limitations

- Western blot has limited temporal resolution (Jha et al., 2016).
- For live cell imaging, a humidity and temperature-controlled microscope unit are required (Gökerküçük et al., 2020).
- TEM is not possible with very low cell numbers, and collaboration with specialized units is required (Vincent et al., 2016).
- Flow cytometry has limited temporal resolution.
- SCENITH has limited temporal resolution (Argüello et al., 2020).
- Seahorse requires an expensive instrument, has no single-cell resolution, and is not possible with very low cell numbers (van der Windt et al., 2016).
- Metabolomics has a limited temporal resolution, and usually, collaboration with specialized units is required (Jang et al., 2018).

An interesting example of a metabolic–functional link in keratinocytes (KCs) comes from a study revealing differential glucose requirements in skin homeostasis and pathology (Zhang et al., 2018). Homeostatic KCs were able to compensate for the absence of the GLUT1 transporter, the main mediator of glucose import into these cells. However, in the context of injury and inflammation, KCs were severely affected by GLUT1 deficiency, and in murine psoriasis-like disease, GLUT1 inhibition improved pathology. Thus, the authors conclude that GLUT1 is selectively required for injury- and inflammation-associated KC proliferation and that its inhibition offers a treatment strategy for psoriasis.

Glycolysis, even in the presence of oxygen, a phenomenon reported around 100 years ago (Warburg effect) (Warburg et al., 1927), is a hallmark of cancer cells. As exemplified in melanoma, more recent data, however, show highly complex and dynamic metabolic adaptations and interactions between cancers cells and the tumor microenvironment, involving the simultaneous upregulation of glycolysis and OXPHOS (metabolic symbiosis) (reviewed, for instance, in Kumar et al. [2021]). Such metabolic adaptations offer great therapeutic potential.

For researchers moving into the field, a key challenge is the complexity of cellular metabolism. Furthermore, techniques employed to study metabolism and its effect on cellular functions may be new, may be sometimes complicated, and may often be difficult to interpret. In this article, we describe the common techniques used, with a special focus on those that can independently be performed by most laboratories. Nevertheless, we also provide insights into the fascinating field of metabolomics, which may require collaboration with companies, core facilities, or more specialized laboratories.

Studying mitochondria as a central metabolic hub

Mitochondria are dynamic organelles, adapting their mass and morphology to intrinsic metabolic needs or extrinsic microenvironmental cues. Western blot analysis of different proteins localized to the OMM and IMM as well as the mitochondrial matrix (e.g., TOM20, ETC complexes subunits, or TCA cycle enzymes) provides a basic set of structural and functional information. This simple technique was, for instance, applied by Weiland et al. (2018) investigating the role of mtDNA in skin aging. Their mouse model, characterized by massive epidermal mtDNA depletion, showed imbalanced stoichiometry of mitochondrial respiratory chain complexes (Figure 1a) (Weiland et al., 2018). The mitochondrial alterations caused unique cytokine expression leading to a severe inflammatory phenotype. Thus, Weiland et al. (2018) conclude that their data unravel a previously unknown link between an imbalanced stoichiometry of mitochondrial respiratory chain complexes and skin inflammation.

Analysis of ETC subunits can be performed under denaturing conditions, showing the protein levels of the different subunits, or in native states (without denaturing agent present in the lysis buffer and gels) (Jha et al., 2016). This native analysis can reveal the quaternary organization of the ETC subunits in supercomplexes, which modulate the efficiency of OXPHOS.

Morphologically, mitochondria can be organized in fragmented units or in a network of elongated filaments inside the cell. Mitochondrial shape is defined by a cohort of so-called mitochondria-shaping proteins, including, among others, MFN1, MFN2, and OPA1 (regulating fusion) and DRP1, MFF, and FIS1 (controlling fragmentation). Their protein levels as well as post-translational modifications (e.g., phosphorylation) can reveal information about the upstream cellular signaling instructing mitochondrial morphology. OPA1 has a

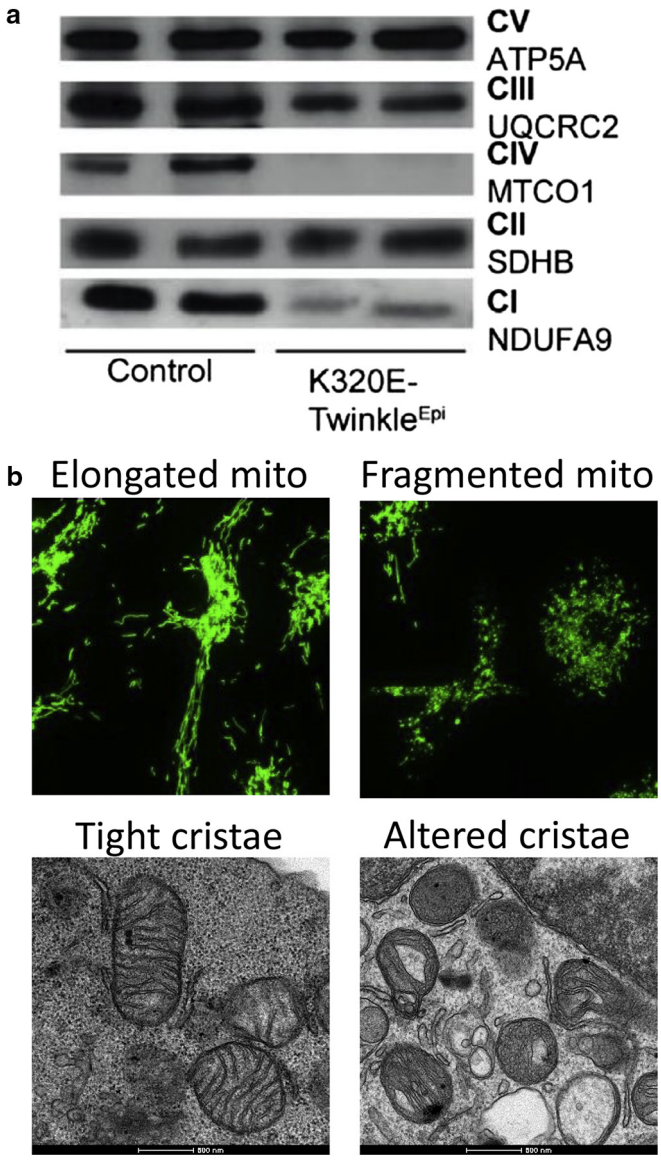

Figure 1. Analyses of mitochondria by western blotting and confocal and transmission electron microscopy. (**a**) In this example, mitochondria of the epidermis were analyzed by western blotting. Mice expressing a mutant epidermal mitochondrial helicase (K320E-Twinkle), leading to pronounced mtDNA depletion, show mitochondrial alterations in the epidermis, as indicated by western blot analyses of respiratory chain units (Weiland et al., 2018). (**b**) Bone marrow–derived mouse macrophages isolated from PhAM mice were polarized to an alternative-activated phenotype by culturing them for 7 days with 20 ng/ml M-CSF and for 1 additional day with 20 ng/ml IL-4. Representative confocal images (top panel) and representative EMs (bottom panel) before and after 1-hour treatment with FCCP, a drug that depolarizes the mitochondrial membrane potential. FCCP was used as a control of depolarisation in this experiment because of its known profragmentation and procristae remodeling effect. The images depict elongated and fragmented mito (confocal microscopy) as well as tight and remodeled cristae (EMs). In **b**, we show previously unpublished images. EM, electron micrograph; M-CSF, macrophage colony-stimulating factor; mito, mitochondria; PhAM, photoactivatable mitochondria.

central role in regulating not only mitochondrial shape but also mitochondrial cristae structure that, on one side, controls apoptosis (through cytochrome c sequestration or release) and, on the other side, metabolism (by favoring the quaternary organization of ETC subunits in supercomplexes). OPA1 is synthesized in different isoforms and cleaved by proteases generating a characteristic multiple band pattern on a western blot. Accumulation of short over long isoforms of OPA1 may suggest altered cristae structure and cytochrome c release.

Mitochondrial morphology can be visualized by immunofluorescence. Examples of this include using confocal microscopy to assess mitochondria in cells from photoactivatable mitochondria mice, which express a photoactivatable GFP protein (Figure 1b) localized to mitochondria, or through staining of mitochondrial proteins such as TOM20 (Gökerküçük et al., 2020). In Figure 1b, bone marrow–derived macrophages were polarized to an alternatively activated phenotype. The mitochondrial morphology of these macrophages was analyzed by confocal microscopy before and after treatment with the depolarizing agent FCCP. It should be noted that acquisition of mitochondrial networks in the entire z-axis of a cell or tissue of interest, followed by z-stack reconstruction, is necessary to avoid morphological artifacts generated by single plane analysis of the cell volume.

When it comes to mitochondrial ultrastructure, detailed analysis of mitochondrial cristae can be performed using transmission electron microscopy (TEM) or tomography. A tight cristae structure indicates mitochondrial fitness, whereas swollen, balloon-like, remodeled cristae (Figure 1b) denotes either mitochondrial damage or cells not relying on mitochondrial respiration for their metabolic and survival needs (Vincent et al., 2016).

Whereas western blot analysis is the cheapest and quickest way to assess some mitochondrial properties, immunofluorescence adds single-cell resolution, and TEM zooms in on the ultrastructure of a single mitochondrion. Altogether, analysis of mitochondrial protein levels, morphology, and ultrastructure complements each other and contributes to a general characterization of mitochondrial metabolic and functional status.

Flow cytometry to investigate metabolism

Flow cytometry represents a quick and easy method to detect and quantify protein levels and resolve cellular metabolic states at a single-cell level. It lends itself as a simple first approach and can be applied to characterize even rare cell populations in complex mixtures. A common approach uses fluorescent dyes that specifically stain different cellular organelles on the basis of the chemical compositions of their membranes. As such, MitoTracker and LysoTracker dyes can be used to stain and quantify mitochondria and lysosomes, respectively. Similarly, dyes sensitive to changes in pH or electric potential can be used as proxies of the metabolic activities of certain organelles. Among those, tetramethylrhodamine, methyl ester (TMRM) and JC-1 measure the electrochemical potential across the IMM, which is indicative of active mitochondrial OXPHOS. A basic example of such staining is shown in Figure 2. Mouse T cells, when activated, increase their mitochondrial mass, as indicated by increased MitoTracker staining, and display an increased potential across the IMM as indicated by a higher TMRM signal. Fluorescent analogs of major energy sources, such as the glucose analog 2-NBDG or Bodipy-labeled fatty acids can be used to assess the uptake of these metabolites. The combination of these dyes with staining for canonical surface

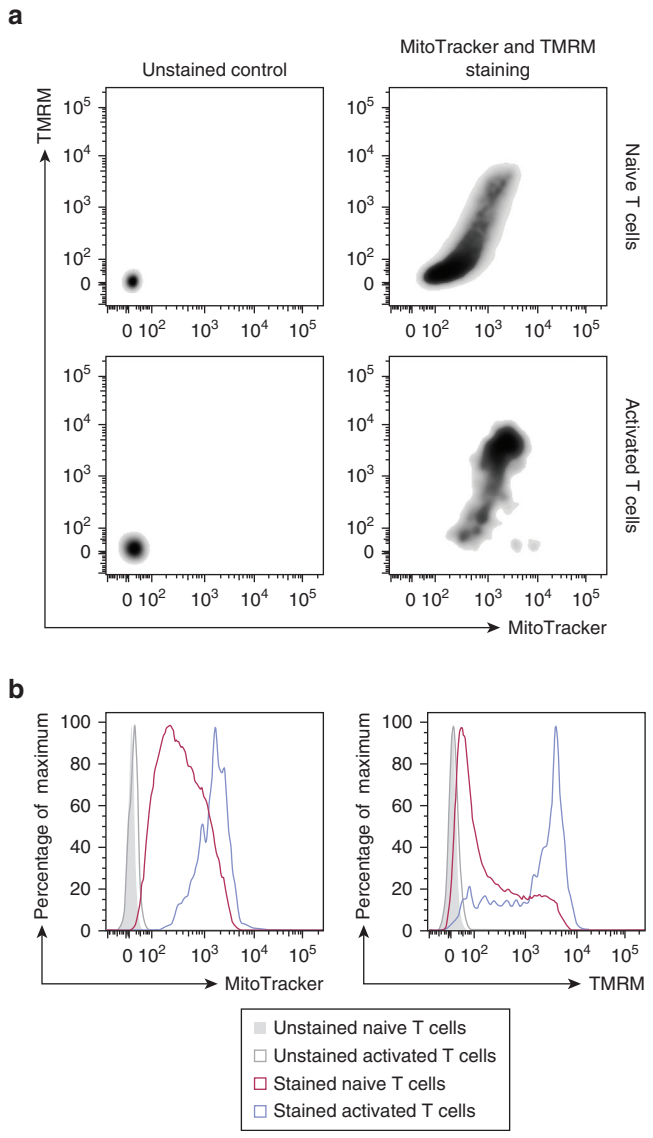

Figure 2. Flow cytometric analysis of cellular metabolism. (**a**) Density plots showing mitochondrial content (measured with MitoTracker green) and mitochondrial transmembrane potential (measured by TMRM) of naive and activated T cells isolated from lymph nodes of unchallenged mice. (**b**) Histograms representing the same data as in **a**. Gray traces show the unstained controls, and blue and red traces show MitoTracker green and TMRM staining, respectively, in naive and activated T cells. **a** and **b** show previously unpublished graphs. TMRM, tetramethylrhodamine, methyl ester.

markers further allows the specific analysis of distinct cell populations.

Fluorophore- or heavy metal isotope–conjugated antibodies against metabolic enzymes, transporters, and regulators can be used in combination with flow cytometry or cytometry by time of flight (Matos et al., 2017; Naderi-Azad et al., 2021) to quantify the levels of those proteins at a single-cell level and thereby give a snapshot of the metabolic status of a given cell (Artyomov and Van den Bossche, 2020). This, in combination with single-cell RNA sequencing, can also allow for conclusions regarding the post-transcriptional regulation of metabolism.

A recent development, SCENITH (which stands for single-cell energetic metabolism by profiling translation inhibition), allows for functional testing of the dependency of cells on different metabolic pathways (Argüello et al., 2020). It is based on the incorporation and subsequent detection of the nucleoside antibiotic puromycin into nascent protein chains as a way of quantifying the rate of protein translation. Because translation is highly dependent on cellular ATP pools, this allows for the assessment of the overall metabolic activity of a cell. In combination with the use of specific inhibitors of different metabolic pathways, this technique probes the relative contributions and utilization of different energy sources. In that regard, SCENITH is similar to the Seahorse Analyzer, which will be discussed in the next section, but does not require the purification of cell populations or major equipment beyond a flow cytometer. It can therefore be directly applied ex vivo, for example, on blood samples.

Although the single-cell resolution of flow cytometry–based approaches is a great advantage, most of the mentioned techniques only represent snapshots in time and do not provide temporal resolution. However, measurements after different lengths of time or newer developments, such as SCENITH, allow for the functional characterization of metabolism.

The Seahorse Analyzer and the functional analysis of cellular metabolism

The two key pathways involved in the production of ATP, the main energetic currency within the cell, are glycolysis and OXPHOS. The engagement of these metabolic pathways can be quantified by measuring the secretion of lactate and oxygen consumption, widely viewed as proxies of their respective activity. Tools to quantify lactate accumulation in culture medium are commercially available as well as are techniques capable of measuring oxygen utilization by live cells such as Clark-type oxygen electrodes or the Oroboros oxygraph (Oroboros Instruments, Innsbruck, Austria). Another popular tool that combines the assessment of lactate secretion and oxygen consumption to ultimately quantify energy metabolism is the Seahorse Extracellular Flux Analyzer (Agilent Technologies, Santa Clara, CA) or Seahorse for brevity, which allows for real-time functional quantification of cellular energetics on a relatively low number of live cells (van der Windt et al., 2016). On one hand, Seahorse measures the accumulation of protons in the extracellular medium (extracellular acidification rate) used as a readout of the secretion of lactate. Seahorse also quantifies the oxygen consumption rate (OCR), which is essential for ETC function. The controlled delivery of metabolism-modifying drugs allows for the kinetic analysis of energy metabolism on different treatments. For instance, mitochondrial utilization can be interrogated using a Mito Stress Test. OCR is recorded before and after treatment with oligomycin, an inhibitor of the complex V, to quantify the oxygen utilization linked to ATP synthesis. Cells are then treated with FCCP, an ionophore that dissipates the proton gradient across the IMM. To counteract the dissipation of the proton gradient, cells maximally engage the ETC and, ultimately, oxygen consumption. The differential of OCR before oligomycin and after FCCP treatments is defined as spare respiratory capacity, and it estimates the ability of a cell to meet an energetic challenge. Cells are finally treated with rotenone and antimycin A, inhibiting complexes I and III,

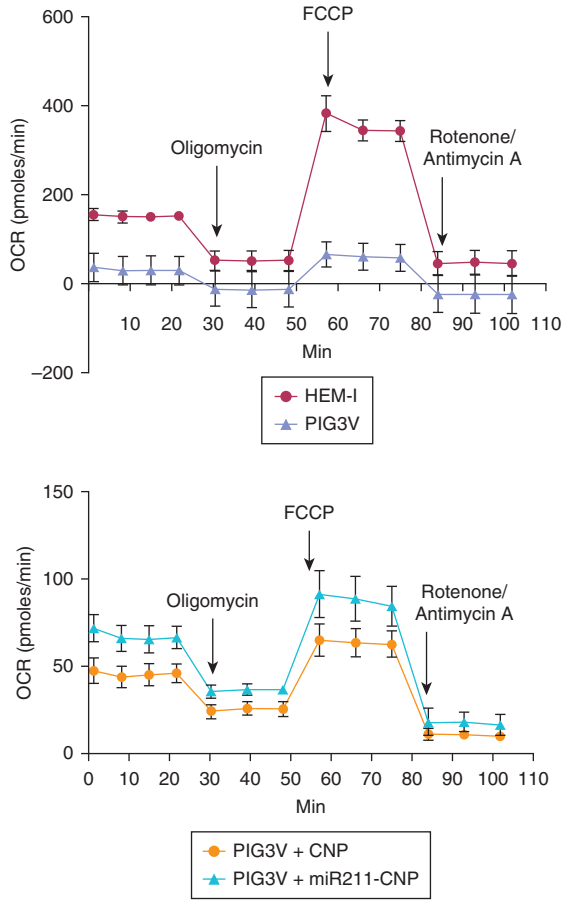

Figure 3. Analyses of cellular metabolism by Seahorse. Sahoo et al. (2017) studied metabolic control of vitiligo development and progression using a human vitiligo and a normal melanocyte cell line as models. A Mito Stress Test (see text for details) was performed on the human vitiligo (PIG3V) and control (HEM-1) melanocytes (top). Vitiligo melanocytes were characterized by a reduced basal OCR and reduced SRC, indicating a reduced capacity of mitochondrial respiration (OXPHOS) (Sahoo et al., 2017). In the same paper, the authors show a Mito Stress Test of the PIG3V cell line treated with a miR-211–coated cerium oxide nanoparticle (miR211-CNP), which partially restores mitochondrial functionality. Note that the data in the top graph show some negative values. To avoid this, many investigators normalize their values to, for instance, basal OCR values. On normalization, OCR values are shown relative to the respective basal OCR. Normalized OCR values can be compared across experimental conditions that would otherwise show different basal OCR, thus easing interpretation and quantification of SRC (see text for details). However, plotting of absolute OCR values carries additional information compared with that of normalized values. Absolute values show the basal OCR rate that is dependent on the intrinsic metabolic activity of cells when equal cell numbers are compared. In our opinion, the negative nature of such values should not be overinterpreted. It is indeed very likely that these values are negative on the treatment of cells with oligomycin owing to the low starting values of basal OCR of PIG3V cells. Normalization would have overcome the issue of negative values and yet would also have hidden the differences in basal OCR between the analyzed samples. min, minute; OCR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; SRC, spare respiratory capacity.

respectively, which block the electron flow through the ETC and prevent mitochondria-dependent oxygen consumption. A Mito Stress Test was, for instance, used by Sahoo et al. (2017). The authors studied metabolic control of vitiligo development and progression using a human vitiligo and a normal melanocyte cell line as models (Sahoo et al., 2017).

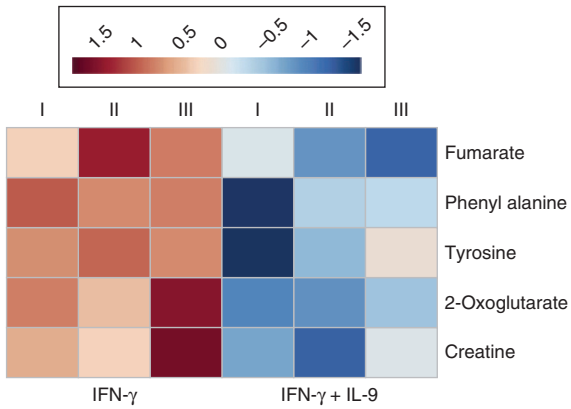

Figure 4. Analyses of human KCs by metabolomics. A recently published multiomics approach was used to study how IL-9 regulates metabolism in human primary KCs. One piece of the puzzle, defining IL-9 as a master regulator of KC metabolic reprogramming, was derived from metabolomics. Shown is a heat map displaying the significantly altered metabolites in primary KCs after stimulation with IL-9 plus IFN-γ versus IFN-γ alone (Marathe et al., 2021). KC, keratinocyte.

One finding of their study was a reduced oxidative capacity in vitiligo melanocytes compared with that in normal melanocytes shown by Seahorse analyses (Figure 3). These data, along with changes in lipid and metabolite profiles, and increased ROS production observed in vitiligo cells appeared to be partly due to abnormal regulation of microRNA-211 and its target genes, representing potential biomarkers and therapeutic targets in this skin disease.

In Seahorse assays, it is also possible to design drug combinations to test the contribution of various metabolic pathways, such as glycolysis or fatty acid oxidation, in energy production. Although representing a state-of-the-art technique to investigate cellular metabolism, Seahorse analysis has some limitations. Conceptually, it might oversimplify cellular energetics. It requires a dedicated instrument (controller) that might not be available in every laboratory or facility. Moreover, the analysis of rare cell populations (e.g., <50,000 T lymphocytes per well) is challenging. However, because Seahorse sensitivity was refined during the transition from 24- to 96-well plates, further miniaturization of the format might allow for an analysis of such cell populations. Of note, the Seahorse analysis relies on excellent cell viability to obtain robust and reproducible data. The isolation of cells from tissue samples such as the skin may fall short of meeting such requirements owing to the extensive and harsh procedures the cells are exposed to during the isolation.

Omics approaches in metabolic research

Metabolomics, defined as the comprehensive study of metabolites present in a given biological system, is the newest of the global omics-based platforms. Over the past 20 years, advances in high-resolution mass spectrometry and computational analysis platforms have rapidly increased coverage of the metabolome. The Human Metabolomics Database is continuously expanding and currently records over 8,000 metabolites detected in human samples (e.g., from blood, urine, saliva) and theoretically predicts over 90,000

endogenous metabolites. A key advantage of metabolomics as a method to probe cellular metabolism is the ability to take a snapshot of many metabolites simultaneously (Figure 4). This can take the format of a targeted analysis, for example, to measure multiple metabolites in a pathway of interest, or of an untargeted analysis, for example, to discover changes in metabolites that can be mapped to metabolic pathways. Within each of these formats, labeling cells or organisms with stable isotope–labeled substrates and measuring the rate of incorporation into a metabolic product over time can achieve valuable insight into metabolic pathway dynamics (Jang et al., 2018).

A typical metabolomics experiment comprises three key stages: (i) metabolite extraction, (ii) metabolite measurement, and (iii) data analysis. Metabolite extraction protocols vary on the basis of the physicochemical properties of the metabolites the user intends to extract. An important example of this is the different extraction methods for hydrophilic polar molecules (e.g., carboxylic acids of the TCA cycle and sugars) or hydrophobic nonpolar molecules (e.g., fatty acids and membrane lipids). Therefore, a limitation of metabolomics is that multiple extraction methods are required to achieve broad coverage of the metabolome. Metabolite measurement involves the chromatographic separation of metabolites, which are then

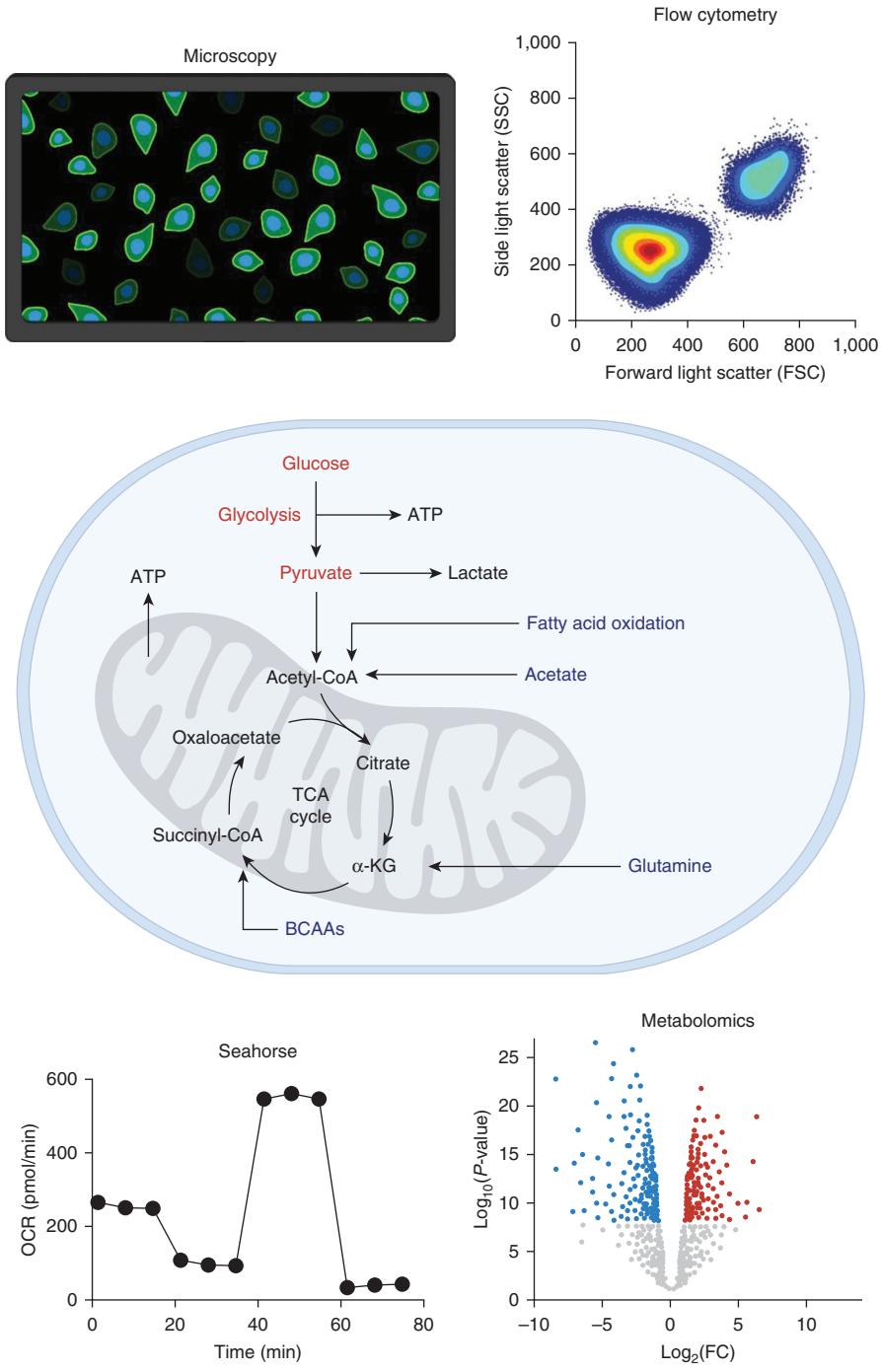

Figure 5. Summary: A comprehensive analysis of core metabolic functions. Core energy metabolism, shown in the center, can be analyzed by many different methods, including microscopy (upper left corner), flow cytometry (upper right corner), Seahorse assays (lower left corner), or metabolomics (lower right corner). Under consideration of individual assay advantages and disadvantages, together, such tools can provide detailed information on cellular metabolism. α-KG, α-ketoglutarate; acetyl-CoA, acetyl-coenzyme A; ATP, adenosine triphosphate; BCAA, branched-chain amino acid; FC, fold change; FSC, forward scatter; min, minute; OCR, oxygen consumption rate; SSC, side scatter; succinyl-CoA, succinyl-coenzyme A; TCA, tricarboxylic acid.

coupled to a mass spectrometer for detection and quantification. Chromatography and mass spectrometry instruments require substantial expertise and maintenance to operate and are often managed as a core facility. Data analysis workflows involve picking peaks that correspond to metabolites from background peaks caused by contaminants. Metabolites can be identified by accurate mass and by matching to the retention time (determined by the chromatography) and/or the fragmentation pattern (measured using tandem mass spectrometry) of known standards.

Future advances in metabolomics aim to increase the numbers of identified metabolites per experiment. Although state-of-the-art metabolomics methods can measure over 1,000 features, this routinely equates to 100 annotated metabolites. This differs from other omics technologies (e.g., proteomics) where identification is not a limitation. Studies to interlink metabolomics with other omics technologies will offer deeper insight into metabolic regulation. A recently published multiomics approach was used by Marathe et al. (2021) to study how IL-9 regulates metabolism in human primary KCs. A finding derived from metabolomics was that IL-9 reduced the production of TCA cycle intermediates in human primary KCs (Figure 4). Integration with multiomics data, systems-level analysis, and confirmatory biochemical assays revealed that IL-9 leads to increased glucose consumption and redirection of metabolic flux toward lactate. Thus, the authors conclude that IL-9 is a master regulator of KC metabolic reprogramming.

New technologies such as imaging mass spectrometry can measure metabolic profiles on a single-cell level and may be studied alongside single-cell RNA sequencing to capture metabolic heterogeneity (Artyomov and Van den Bossche, 2020). Finally, isolation of organelles before metabolite extraction can be used to understand the spatial organization of metabolism within the cell.

Conclusion

Metabolism in homeostasis and disease is a rapidly emerging research field. Clearly, scientists studying all areas of dermatology will increasingly explore metabolism in the context of their specific interests. In addition to the references given in the main text, examples of published, dermatology-related works using the techniques described in this paper can be found in Supplementary Table S1. The series of techniques described in our article, which can be performed in almost all wet laboratories, allows for an adequate, state-of-the-art characterization of cellular metabolic states (Figure 5). Moreover, collaboration with specialists on metabolomics and transcriptomics provides a greater chance for deeper insights into the coordination of cellular metabolic pathways.

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MULTIPLE CHOICE QUESTIONS

1. Which are the core pathways for cellular ATP synthesis?
   A. Glycogenolysis and glycolysis
   B. Gluconeogenesis and glycolysis
   C. Fatty acid oxidation and glycogenolysis
   D. Glycolysis and OXPHOS
2. Which proteins regulate changes in mitochondrial morphology?
   A. ETC subunits
   B. Mitochondria-shaping proteins
   C. TCA enzymes
   D. Nuclear transcription factors
3. What cellular process does SCENITH measure?
   A. Glycolysis
   B. DNA replication
   C. Transcription
   D. Translation
4. What cellular metabolic parameters are measured during a Seahorse analysis?
   A. Secretion of lactate and oxygen consumption.
   B. Proton gradient across the IMM and glucose consumption.
   C. Spare respiratory capacity and oxygen secretion.
   D. Reduced nicotinamide adenine dinucleotide (NADH) synthesis and glucose uptake.
5. Which of the following classes of molecules could be defined as metabolites?
   A. Amino acids
   B. mRNA
   C. DNA
   D. Proteins

CONFLICT OF INTEREST

ELP is a founder of Rheos Medicines and a science advisory board member of Immunomet Therapeutics. The remaining authors state no conflicts of interest.

ACKNOWLEDGMENTS

This work was funded by the Deutsche Forschungsgemeinschaft (CRC829 to MF) and the Barth Syndrome Foundation Idea Grant (to MC). MF and ELP hold faculty positions. MV, MAS, and JEH are postdocs, and MC is an independent investigator.

AUTHOR CONTRIBUTIONS

Conceptualization: MF, MV, MAS, JEH, MC; Funding Acquisition: MF, MC; Investigation: MV, MC; Resources: ELP; Visualization: MF, MV, MAS, JEH, MC; Writing - Original Draft Preparation: MF, MV, MAS, JEH, MC, ELP; Writing - Review and Editing: MF, MV, MAS, JEH, MC, ELP.

DISCLAIMER

The funders had no role in the design, decision to publish, or preparation of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to this paper. Teaching slides are available as supplementary material.

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DETAILED ANSWERS

1. **Which are the core pathways for cellular ATP synthesis?**

CORRECT ANSWER: D. Glycolysis and OXPHOS

Almost all cellular ATP is derived from glycolysis, which does not require oxygen, and oxygen-dependent OXPHOS (mitochondrial respiration).

2. **Which proteins regulate changes in mitochondrial morphology?**

CORRECT ANSWER: B. Mitochondria-shaping proteins

Mitochondrial morphology is regulated by so-called mitochondria-shaping proteins. Among them, OPA1, MFN1, and MFN2 control mitochondrial fusion and DRP1, MFF, and FIS1 control fragmentation.

3. **What cellular process does SCENITH measure?**

CORRECT ANSWER: D. Translation

SCENITH measures the rate of protein translation by quantifying the incorporation of puromycin into nascent protein chains.

4. **What cellular metabolic parameters are measured during a Seahorse analysis?**

CORRECT ANSWER: A. Secretion of lactate and oxygen consumption

The Seahorse analysis measures the production of lactate, readout of anaerobic glycolysis, and oxygen consumption, the final electron acceptor in the ETC.

5. **Which of the following classes of molecules could be defined as metabolites?**

CORRECT ANSWER: A. Amino acids

A metabolite is any small molecule resulting from metabolic reactions.

Supplementary Table S1. Examples of Dermatology-Related, Published Works Studying Cellular Metabolism by the Techniques Described in the Main Article

Technique	References
Western blot	Schilf et al., 2021 Wang et al., 2021
Imaging	Kim et al., 2020 Monteleon et al., 2018 Yu et al., 2020
TEM	Wang et al., 2021 Yu et al., 2020
Flow cytometry	Schilf et al., 2021 Wang et al., 2021 Yu et al., 2020
Seahorse	Kim et al., 2020 Monteleon et al., 2018 Russell et al., 2017 Rybchyn et al., 2018 Schiffmann et al., 2020 Schilf et al., 2021 Tan et al., 2019 Vogel et al., 2019 Wang et al., 2021 Wickersham et al., 2017
Metabolomics	Chen et al., 2021 Kamleh et al., 2015 Kim et al., 2020 Schiffmann et al., 2020 Schilf et al., 2021 Sinclair et al., 2021 Vogel et al., 2019

Abbreviation: TEM, transmission electron microscopy.

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