Taking metabolism on the road

Extracellular vesicles (EVs) are a class of secreted membrane particles capable of transferring biological molecules between cells. Metabolomics measurements indicate that isolated EVs also have autonomous metabolic enzyme activities, including the unexpected identification of endogenous human asparaginase activity.

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The exchange of molecules between tissues by EVs has emerged as a modifier of physiology. Recently, understanding of the functional consequences of EVs has expanded, implicating EVs from various cells in both supportive and antagonistic roles that affect diverse processes including immune responses, cancer, and the nervous system. Typically, EVs are thought to mediate their effects through direct transfer of proteins, RNAs, and small molecules from a donor cell into the cytosol of a recipient cell. In this issue, Iraci et al. describe a new role for EVs in altering metabolism, adding to the list of methods by which EVs affect organismal homeostasis.

Each tissue exhibits metabolic activities that are specialized and tightly controlled to accomplish its particular function. These metabolic functions are often interdependent, with specific molecules being produced by one tissue and released into circulation to act at another tissue. The excretion of EVs is another method by which tissues can alter the metabolism of nearby and distant tissues. Direct transfer of metabolic enzymes, RNAs, and metabolites by EVs can alter the metabolic phenotype of recipient cells (Fig. 1). However, it is unknown whether EVs can also function autonomously to modify physiology.

EVs contain a diverse set of intracellular proteins, including metabolic enzymes; thus, Iraci et al. sought to determine whether EVs could independently alter the metabolites in their environment. Purified EVs from neural stem/progenitor cell (NSC) cultures were incubated in cell culture media, and metabolite levels were measured by MS. Interestingly, EVs were sufficient to alter metabolite levels, resulting in notable increases in glutamate, GABA, and aspartate, and a decrease in asparagine (Fig. 1). Importantly, heat inactivation of EVs abolished these changes, indicating that enzymatic activity was required to modulate metabolite levels. This experiment demonstrates that EVs can be metabolically active before they reach their target tissues and are capable of enzymatically modifying their metabolic environment.

Of the metabolic activities observed, the consumption of asparagine and production of aspartate was particularly noteworthy, because it suggested that NSC-derived EVs harbor asparaginase activity, an enzyme thought to be absent in most mammals. Conversion of isotope-labeled asparagine into labeled aspartate by NSC-derived EVs confirmed asparaginase activity, prompting an effort to identify the enzyme responsible. Mouse and human genomes encode two asparaginase-like proteins, Aspg and Asrgl1, although assessments of the recombinant enzyme activities of both proteins have shown low affinity for asparagine. Through expression profiling of NSCs and modification of protein levels, Iraci et al. present data that Asrgl1 has asparaginase activity at physiological asparaginase concentrations in EVs and parental NSCs, but not as a recombinant enzyme. Thus, some human and mouse tissues may have conditions that activate asparaginase function mediated by Asrgl1, and this mechanism will be important to characterize. Additionally, this finding demonstrates how EVs can potentially affect organismal metabolism: the unique metabolic activity of a tissue is packaged into EVs and can be used to modify circulating metabolite levels and destination microenvironments (Fig. 1).

The finding of asparaginase activity, and the identification of Asrgl1 as the catalyst of that activity in EVs, has potential implications for cancer therapy. Bacterial asparaginases are used to treat acute lymphoblastic leukemia, but their use can be limited by immunological side effects. Use of activated human Asrgl1 in EVs is an attractive therapeutic strategy, as it may provide the clinical benefit of asparaginase treatment without the immunogenic effects of administering a bacterial enzyme.

Figure 1 | Mechanisms by which extracellular vesicles (EVs) can alter organismal metabolism. Donor cells (purple) release EVs, whose metabolic enzyme cargo can alter metabolite levels local to the donor cells, in circulation (red), and in the microenvironment of target tissues (orange). EVs can also modify intracellular metabolism of recipient cells by delivery of macromolecules and metabolites. Examples of metabolic activities in neural stem/progenitor cell-derived EVs by Iraci et al. are also shown. Asrgl1 can convert asparagine (Asn) to aspartate (Asp), and glutaminase (Gls) can convert glutamine (Gln) to glutamate (Glu). In complex biological fluids, other metabolic enzymes likely present in EVs could perform other reactions to alter nutrient availability, as represented by the bottom line (X and Y).
Further work is needed to catalog the full range of autonomous metabolic reactions of EVs in physiological contexts and to determine how metabolism of EVs resembles and differs from that of parental cells. EVs may vary from parental cells by selectively incorporating proteins. Indeed, some metabolic proteins have been reported to be enriched in EVs in some contexts. However, even if EV metabolic enzymes were a microcosm of their parental cytoplasm, the metabolic activities of EVs would still likely differ from those of the parental cells. Because EVs lack the organelles present in intact cells, their metabolic enzymes may be constrained to reactions that do not require consumption of coenzymes normally replenished in reactions compartmentalized into organelles. As a prominent example, the mitochondrion replenishes cellular NAD⁺ and ATP, and, without regeneration of these molecules, some EV-associated metabolic enzymes may be limited in the number of catalytic cycles they can perform. Interestingly, the three metabolites most increased by EVs observed by Irci et al., result from glutaminase, asparaginase, and glutamate decarboxylase reactions, which do not require consumption of coenzymes. Regardless, the specific metabolic activities observed in defined media likely represent only a subset of the numerous reactions that could occur in metabolically complex biological fluids. Future work testing the multitude and magnitude of metabolic alterations in relevant biological fluids will be important to determine the full extent that EVs can autonomously alter metabolism.

References

Competing financial interests
The author declares no competing financial interests.

LOCKING DOWN METABOLISM

An allostERIC inhibitor of Mycobacterium tuberculosis tryptophan synthase—an enzyme that is nonessential for in vitro growth—has potent antimicrobial activity, revealing a potentially expanded target list for antimicrobials and greater chemical space for new inhibitors.

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Conventional wisdom has deemed targeting enzymes that are nonessential for in vitro growth of microbes to be risky business, given the desire for killing bugs in culture. Acceptable drug targets have generally been considered to necessarily be products of essential genes, preferably ones without human counterparts. Despite the fact that M. tuberculosis is an obligate human pathogen, whose only substantial ecologic niche is the human body, only 614–1,487 of its ~4,000 genes are thought to be essential for in vitro growth, resulting in a drug target list that is a disappointing limited2,3. Wellington et al. now describe the discovery of an allostERIC inhibitor of a key metabolic enzyme from M. tuberculosis—tryptophan synthase (TrpAB)4. By demonstrating that an enzyme nonessential for in vitro growth is indispensable during in vivo infection, the work expands the arena of valuable drug targets to genes of central metabolism that may have been previously dismissed due to in vitro nonessentiality.

Using a diversity-oriented synthetic library, the study identified a chiral azetidine, BRD4592, that is active against M. tuberculosis, with an MIC₉₀ (the minimal concentration needed to inhibit 90% of bacterial growth) of 3 μM. Mutants resistant to BRD4592 showed substitutions in TrpAB, the heterotetrameric, bifunctional enzyme that catalyzes the last two steps in the biosynthesis of tryptophan, which is one of the costliest amino acids produced by prokaryotes (>70 ATP molecules required per tryptophan synthesized) and is tightly regulated at multiple levels. biochemical analysis showed that BRD4592 inhibited both reactions catalyzed by TrpAB, with IC₉₀ values in the nanomolar range, and indicated that BRD4592 functions as a mixed-type inhibitor of the enzyme’s two catalytic activities. Subsequent crystallographic studies found that BRD4592 binds outside of the active sites of TrpAB, instead occupying a cavity in the subunit interface, blocking a tunnel through which free indole is believed to pass. The drug forces the enzyme into a closed active conformation, preventing catalytic cycling and promoting product trapping, essentially locking down the enzyme in an inactive state.

Going against the grain of conventional wisdom, the work of Wellington et al. reveals central metabolism to be a potentially fertile pasture for drug discovery. TrpAB, encoded by genes that may be mutated without loss of in vitro growth, was potently inhibited by this small molecule derived from a synthetic library with considerable chiral diversity. Enzymes of central metabolism that are required under specific nutrient-limiting conditions are thought to have evolved under less evolutionary pressure than gene products that are essential under nutrient-rich conditions. Therefore, these processes may be less prone to mutational resistance, which is an attractive attribute in the current era of evolving resistance of our current antimicrobial armamentarium that largely targets essential processes such a cell-wall, DNA, and RNA biosynthesis (Fig. 1a).

A second key demonstration of the study is the power of allostERIC inhibitors. The finding that a non-natural product is able to potently inhibit TrpAB by binding outside of the enzyme’s active sites underscores the fact that the chemical space available for inhibition may be far greater that that accessible by active site inhibitors alone. AllostERIC inhibitors that lock enzymes into fixed conformations may do so at multiple sites over an enzyme’s surface.