S12 – Participant organized symposium — Energetics of disease-causing microorganisms and the potential for drug discovery

S12.11

Targeting bacterial energetics to produce new antimicrobials

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The emergence and spread of drug resistant pathogens and our inability to develop new antimicrobials to combat resistance (phenotypic and genetic) has motivated scientists to consider non-traditional targets where human homologs clearly exist. Cellular bioenergetics is an area showing promise for the development of new antimicrobials, but the success of this area will only emerge by understanding the role of these energetic processes (e.g. respiration and oxidative phosphorylation) under conditions that prevail in host tissues. In this session, we will examine the recent developments in the field suggesting cellular energetics as a target space for the development of new antimicrobials.

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S12.12

The chemical biology of ATP synthase inhibition in mycobacteria

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S12.13

Oxidative phosphorylation — A potential drug target in Mycobacterium tuberculosis

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With the increasing occurrence of drug resistance in diseases including tuberculosis (TB) new drug targets, new lead compounds, and new antimicrobial strategies are all of interest. Recently TMC207/Bedaquiline, a compound that inhibits ATP synthase in mycobacteria, was approved for use in patients with multidrug-resistant or extensively drug-resistant-TB suggesting that oxidative phosphorylation is a vulnerable drug target in mycobacteria. This view is supported by studies from our laboratory and others that suggest menaquinone synthesis (required for electron transport in mycobacteria) is also a valid drug target. Recent data indicates that saturation of a single isoprene unit in the menaquinone of Mycobacterium tuberculosis represents a novel virulence factor for this pathogen. Rv0561c, annotated as a possible oxidoreductase, in the Mycobacterium tuberculosis H37Rv genome and MSMEG1132 in Mycobacterium smegmatis are shown to encode enzymes that increase the mass of menaquinone by two AMU. Mass spectral analysis unambiguously demonstrated that these enzymes did not reduce the aromatic ring moieties, but increased the mass of the isoprenyl side chains by reducing one double bond. Thus, this previously undescribed reductase catalyzes the final step in the synthesis of the predominant form of menaquinone found in mycobacteria and, presumably, other Gram-positive bacteria that synthesize partially saturated
menaquinone. Deletion mutants have reduced oxygen consumption rates and electron transport efficiency, although membrane integrity, cellular ATP levels and rates of ATP synthesis are unaltered as are bacterial growth rates. However, in vitro infection studies using J774A.1 cells and the M. tuberculosis Rv0561c deletion mutant demonstrated that the mutant is unable to survive in macrophage-like cells. Thus, Rv0561c represents a novel, contextually essential enzyme that is a potential drug target in M. tuberculosis. Funded by NIH/NIAID grant # AI049151.

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S12.02
Functional and structural characterization of a Staphylococcus aureus flavohemoglobin
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Flavohemoglobins (flavoHbs) play a key role in bacterial resistance to nitrosative stress and NO signaling modulation. Typically, flavohbts contains three domains: an N-terminal globin domain which harbors a single heme type b and a C-terminal ferredoxin reductase-like FAD- and NAD-binding module. In this presentation, we cloned, expressed and characterized the flavoHb from the opportunistic pathogen, Staphylococcus aureus. The high amino acid sequence homology with Saccharomyces cerevisiae was used to build a model structure by homology modeling showing structural similarities with those of other known flavohbs. Interestingly this high sequence homology did not correlate with the enzymatic and kinetic properties which are much similar to those of Escherichia coli. In vitro and aerobically, the enzyme accepts cytochrome c and oxygen as substrate. Based on this feature, we showed that in Staphylococcus aureus and Rabdona eutropha flavohbts the preferences for cytochrome c and dioxygen depending on the presence of NO dioxygenase inhibitors are different and this is determined by the inhibitor chemical composition. To make progress in understanding the catalytic mechanism of flavohbs we investigated the enzyme properties, the effect of azole antibiotics and the structure-function relationship in comparison to the well-known flavohbts from the non-pathogenic bacteria. The mutation of key residues situated in

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S12.01
Cancer energy metabolism under hypoglycemia
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Most studies on cancer metabolism have been carried out in cells cultured with high glucose (25 mM; hyperglycemia). However, the glucose concentrations range from 5 mM in well blood-irrigated areas to 0.25–2.5 mM in areas within solid tumors that are away from blood vessels. These low glucose levels may induce changes in the cancer cell behavior. Indeed, it is well documented that culturing with low glucose promotes substantial variations in the mRNA levels of a multitude of genes. However, no evidence have been so far provided about the impact that such transcriptional remodeling has on the protein contents and activities of enzymes/transporters and, perhaps more importantly, whether these changes significantly modify pathway fluxes and cellular functions.

In the present study, it was analyzed that the effect of hypoglycemia on the contents of glycolytic and mitochondrial proteins, activities of enzymes/transporters and fluxes of glycolysis and oxidative phosphorylation (OxPhos) in HeLa and MCF-7 tumor cells. One day hypoglycemia (2.5 mM initial glucose) induced increased protein levels of glucose transporters (GLUT 1 and 3 [2–3 fold]) and hexokinase I (HK; 2.3-fold), compared to hyperglycemia. Remarkably, these increases were not accompanied by increases in the total cellular activities (i.e. Vmax) of GLUT and HK; instead, increased affinity of these activities for glucose surged, which may explain the 2-fold increased glycolytic flux under hypoglycemia. Therefore, a change towards greater catalytically efficient isoforms of two of the main controlling steps sufficed to induce increased glycolytic flux.

Hypoglycemia also induced a decrease in the pyruvate dehydrogenase content and increase in the respiratory complex I, with no variation in those of isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. The activities of malate dehydrogenase, fumarase and citrate synthase, as well as the OxPhos flux (i.e., rate of O2 consumption sensitive to oligomycin) and the electrical membrane potential, were not modified by hypoglycemia. Hence, the contribution to the ATP supply by glycolysis increased from 27–35% under hyperglycermia to 44–70% under hypoglycemia, depending on the cancer cell type, and noting that OxPhos contribution was still significant. Therefore, the results indicated that, to effectively diminish the accelerated cancer cell growth, the two energy metabolism pathways should be targeted simultaneously.

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S12.L4
Structure of the trypanosomal alternative oxidase: Opportunities for rational drug design to treat trypanosomiasis
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In addition to haem copper oxidases, all higher plants, some algae, yeasts, molds, metazoans, and pathogenic microorganisms such as Trypanosoma brucei contain an additional terminal oxidase, the cyanide-insensitive alternative oxidase (AOX). AOX is a diiron carboxylate protein that catalyzes the four-electron reduction of dioxygen to water by ubiquinol. In T. brucei, a parasite that causes human African sleeping sickness, AOX plays a critical role in the survival of the parasite in its bloodstream form. Because AOX is absent from mammals, this protein represents a unique and promising therapeutic target. This talk will discuss recent crystal structures of the trypanosomal alternative oxidase obtained in the absence and presence of specific inhibitors. All structures reveal that the oxidase is a homodimer with the non- haem diiron carboxylate active site buried within a four-helix bundle. CAVER protein analysis reveals that there are two hydrophobic cavities per monomer. One cavity, which is perpendicular to the membrane surface, binds both inhibitors and substrate within 4 Å of the active site whereas the second cavity, which is parallel to the membrane surface and also links with the active-site, acts as an oxygen/H2O channel. We suggest that detailed knowledge of the nature of the inhibitor/substrate cavity will lead to a greater rational design of anti-trypanosomal drugs.

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