Abstract

Purines are involved in many biologic processes and are required to maintain stable energy charge, for metabolic regulation, as cofactors in enzymatic reactions and as building blocks in DNA and RNA. Necessary role in purine synthesis is played by *de novo* purine synthesis (DNPS) that is highly active in developing cells. DNPS is complex pathway involving enzymes that assemble into multienzyme complex, purinosome, which facilitates flux of purine intermediates through sequence of ten enzymatically catalyzed reactions. Properly functioning enzymes and purinosome ensure the fast flux of unstable and potentially cytotoxic intermediates resulting in final product IMP, the branch point for AMP and GMP synthesis. The mutations in DNPS genes lead to inherited rare disorders that are accompanied by elevated concentrations of enzyme substrates in body fluids and cells. Lack of a suitable model system to study pathophysiology of DNPS raised the necessity to develop a system mimicking DNPS disorders. HeLa cells with malfunctioning DNPS were characterized on the level of genes, transcripts, proteins, metabolites, and for the presence of the purinosome. Homozygous or compound heterozygous mutations led to the absence of proteins, decreased enzymatic activities, accumulation of enzyme substrates, downregulation of *GART* and *PFAS* genes, and reduced purinosome formation. Dynamic movement of purinosome and core purinosome enzyme GART - mCherry was analyzed in the body wall muscle of nematode *Caenorhabditis elegans*. Additionally, the ability of isotopically labeled DNPS intermediates to enter the cells, engage in DNPS, and their effect on cell viability was tested. Ribosides had a stronger impact on cell viability than ribotides, and intermediates from the second half of DNPS were not cytotoxic in this experimental set up. Within this work, two novel DNPS disorders were discovered: PAICS deficiency and PFAS deficiency.

Key words: *de novo* purine synthesis (DNPS), pathophysiology of DNPS, inherited rare disorders, DNPS disorders, purinosome