

The purinosome is a multienzyme complex composed by the enzymes active in *de novo* purine synthesis (DNPS) that cells transiently assemble in their cytoplasm upon depletion or increased demand of purines [1]. In our previous studies, we proved that various mutations of ATIC and ADSL genes destabilize to various degree purinosome assembly and found that the ability to form purinosomes correlates to clinical phenotypes of individual ADSL patients [2,3]. Therefore, we assume that the assembly of functional purinosomes is fully dependent on the presence of structurally unaffected ATIC and ADSL complexes and presumably also on the presence of all the other DNPS proteins.

We knocked out the expression of purine metabolism enzyme-coding genes (PPAT, GART, PAICS, PFAS, ADSL, ATIC, HGPRT) in HeLa cells using a GeneArt® CRISPR Nuclease Vector with an OFP Reporter Kit and tested the cells for the deletion of proteins, the presence of mutations and the accumulation of DNPS intermediates in the cell lysates and growth media. The cells were seeded in the purine rich or purine depleted medium for the immunofluorescence experiments. Prepared slides were analysed via confocal microscopy. XYZ images were sampled according to the Nyquist criterion using a LeicaSP8X confocal microscope. Images were restored using a classic maximum likelihood restoration algorithm in Huygens Professional Software (SVI). The colocalization maps were created in Huygens Professional Software.

The cells with defect in the DNPS, grown in the PD medium showed disruption or reduced purinosome formation, compared to the controls. Cells with defect in HGPRT formed purinosome not only in the demand of purines, but also in its presence.

We used HeLa cells deficient for specific steps of DNPS to model possible genetically determined defects of DNPS enzymes. We have developed system which does not form purinosome and system forming purinosome in all circumstances. These systems can serve as useful human model systems for the analysis of purinosome assembly and function.

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[2] Baresova V, Skopova V, Sikora J, Patterson D, Sovova J, Zikanova M, Kmoch S., Mutations of ATIC and ADSL affect purinosome assembly in cultured skin fibroblasts from patients with AICA-ribosiduria and ADSL deficiency. *Hum Mol Genet*, **1**, 1534-43 (2012).

[3] Baresova V, Krijt M, Skopova V, Souckova O, Kmoch S, Zikanova M., CRISPR-Cas9 induced mutations along de novo purine synthesis in HeLa cells result in accumulation of individual enzyme substrates and affect purinosome formation. *Mol Genet Meta*, **119(3)**, 270-277 (2016).

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