GLUT10 facilitates dehydroascorbic acid uptake in the endoplasmic reticulum: lessons from arterial tortuosity syndrome

Csilla E. Németh[1], , Paola Marcolongo[2], Angiolo Benedetti[2], Nicoletta Zoppi[3], Marina Colombi[3], Andy Willaert[4], Paul J. Coucke[4], Péter Lőw[5], Pál Gróf[6], Szilvia Nagy[1], Tamás Mészáros[1], Éva Margittai[7], Gábor Bánhegyi[1]

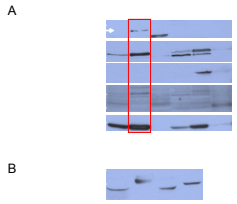
[1]*Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary.*
[2]*Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy.*
[3]*Division of Biology and Genetics, Department of Biomedical Sciences and Biotechnology, Medical Faculty, University of Brescia, Brescia, Italy.*
[4]*Center for Medical Genetics, Ghent University, Ghent, Belgium.*
[5]*Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary.*
[6]*Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary.*
[7]*Institute of Human Physiology and Clinical Experimental Research, Semmelweis University, Budapest, Hungary.*

ABSTRACT

Transport of ascorbate (AA) or its oxidized form dehydroascorbic acid (DHA) into the endoplasmic reticulum (ER) lumen is required for the functioning of luminal AA/DHA dependent enzymes and for the maintenance of the redox/antioxidant homeostasis of the organelle. DHA transport through the ER membrane has been demonstrated, but the molecular mechanism has not been elucidated yet.

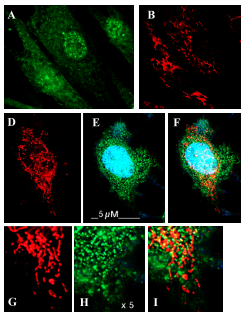
Recent findings verified that mutations in the gene SLC2A10 encoding glucose transporter 10 (GLUT10) are responsible for arterial tortuosity syndrome (ATS), a rare connective tissue disorder characterized by tortuosity, elongation and aneurysms/stenosis of large and middle sized arteries. The pathomechanism of ATS is still an enigma; neither the subcellular localization nor the transported molecule of GLUT10 has been identified. We report here that GLUT10 is a DHA transporter in the ER and nuclear envelope (NE). GLUT10 showed a perinuclear distribution demonstrated by immunocytochemistry in fibroblasts from healthy controls and HepG2 cells, but GLUT10 did not colocalized with mitochondrial markers. Immunoblotting revealed that GLUT10 protein was present in the ER and nuclear fractions of the cells. Transport measurements in cells whose plasma membrane was selectively permeabilized showed that DHA transport and accumulation was markedly reduced in fibroblasts from ATS patients and in GLUT10 silenced immortalized human fibroblasts. Re-expression of GLUT10 in patients' fibroblasts restored DHA transport activity. Measurement of DHA uptake in subcellular fractions of fibroblasts showed that mitochondrial transport was not altered, but ER transport was reduced in patients. GLUT10 protein produced by *in vitro* translation and incorporated into liposomes efficiently transported DHA. Long-term incubation in the presence of AA resulted in a twofold higher steady-state intracellular AA concentration in control fibroblasts. Our data demonstrate that GLUT10 facilitates DHA entry into the ER lumen and probably to the nucleoplasm; the missing function of AA as a cofactor for iron/2-oxoglutarate dependent dioxgenases in these compartments can be a decisive factor of the pathomechanism.

Figure 1. Subcellular localization of GLUT10 in mammalian liver fractions



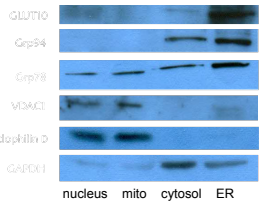
A. Guinea pig liver was fractionated by ultracentrifugation and subcellular organelles were isolated. Different fractions (1. homogenate, 2. ER, 3. cytosol, 4. mitochondria, 5. mitochondria with MAM, 6. MAM) were blotted with GLUT10 antibody, which revealed an accumulation in the microsomal fraction. MAM – mitochondria associated mbr.
B. Indeed, when microsomal fraction was isolated from different species (1. human, 2. mouse, 3. rat, 4. guinea pig), GLUT10 expression could have been detected in all of them.

Figure 2. GLUT10 did not co-localize with mitochondria in fibroblasts or in HepG2 cells



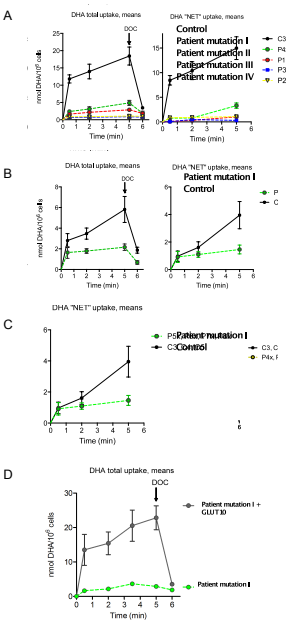
Control fibroblasts (A, B, C) and HepG2 cells (D-I) were decorated with GLUT10 (green), Cytochrome C (red) or both antibodies (panel C, F, I) and acquired by fluorescent microscopy. In both cell types GLUT10 did not show co-localization with mitochondria.

Figure 3. Subcellular fractionation of fibroblast cells revealed a microsomal localization of GLUT10



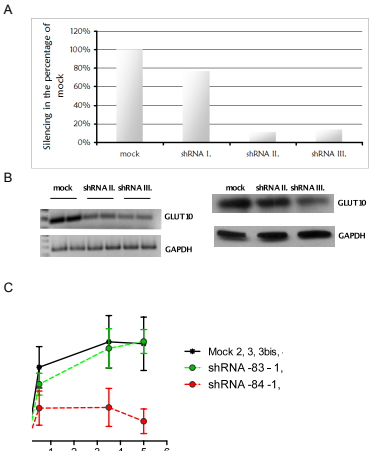
Subcellular fractions were prepared by ultracentrifugation of control fibroblasts. GLUT10 protein was exclusively present in the microsomal (ER) fraction. The appropriateness of the fractionation was checked by organelle-specific antibodies (Grp 94 and 78 – ER, VDAC1 and cyclophilin D– mitochondria, GAPDH - cytosol)
GAPDH-Glyceraldehyde-3-Phosphate Dehydrogenase
VDAC1-Voltage dependent anion channel 1

Figure 4. Ascorbate and dehydroascorbate transport on human control and ATS fibroblasts



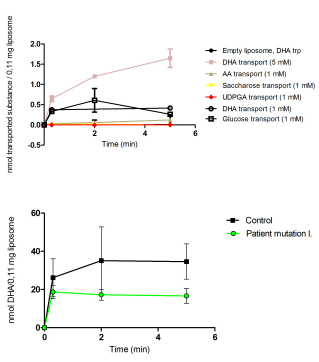
and DHA transport s measured by rapid ation technique on man control fibroblast on fibroblast derived n ATS patients.
After permeabilization the plasmamembrane, A transport was measured and was found to be dramatically decreased in fibroblast derived from ATS patients, indicating a failure of endomembrane-localized transporter.
B. DHA transport decreased in intact ATS fibroblasts as well, with respect to controls.
C. AA transport did not differ between control and patients cells, indicating a DHA-specific transport deficiency.
D. Re-expression of GLUT10 in patients' fibroblasts restored the impaired DHA transport activity in plasmamembrane permeabilized cells.
DOC: deoxycholic acid, added at the end of each measurement to permeabilize the endomembranes.

Figure 5. Silencing of GLUT10 resulted in similar alterations of DHA transport as observed in ATS patients



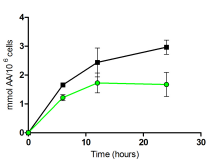
hTERT immortalized human fibroblast cell line was silenced for GLUT10 with lentiviral-based silencing vectors. Stable clones expressing the silencing vector were selected and were checked for the efficiency of silencing. RT-PCR analysis of the stable clones showed that shRNA II. and shRNA III. were effective, which was verified by PCR (Panel B) and Western blot (data not shown) as well. Transport of DHA was measured on plasmamembrane permeabilized cells with rapid filtration technique, and was severely decreased in cells silenced with shRNA III. vector – the one which was most effective in silencing GLUT10 gene.

Figure 6. *In vitro* synthesized GLUT10 protein transported exclusively DHA and glucose



A. *In vitro* translated GLUT10 was inserted into liposomes, and the transport affinity of the protein towards several compounds was measured by rapid filtration technique. The transporter was specific to DHA (and glucose), and the transport of DHA was concentration dependent.
B. Microsomes isolated from control and ATS fibroblasts were inserted into liposomes to check the organelle-specific transport activity of DHA. We found that microsomes isolated from ATS patients showed decreased DHA uptake compared to control microsomes, indicating an ER localization of the protein.

Figure 7. Ascorbate content of ATS fibroblasts reached a lower steady-state level with respect to control



Human control and ATS fibroblasts were incubated with AA for 24 hrs, and the intracellular level of the vitamin was checked in the indicated time points with HPLC. We found that the transport of ascorbate was slower and its steady-state was set up at a lower level comparing with the controls.

Conclusions:

- GLUT10 transporter is expressed in several species and it has a reticular/perinuclear localization in human fibroblasts and liver cells. The previously supposed mitochondrial localization of the protein has been confuted here.
- We proved that DHA transport through the plasma membrane and the endomembrane was decreased on GLUT10 mutant cells and on fibroblasts silenced for GLUT10 transporter. Meanwhile, the transport of ascorbate was unaffected, so patient cells has a specific defect in DHA transport.
- We confirmed the role of GLUT10 in DHA transport by insertion of *in vitro* translated protein into liposomes.