Arterial Tortuosity Syndrome: a vitamin C compartmentation disease? (Grant GGP13167)

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Arterial tortuosity syndrome (ATS, MIM #208050) is a rare autosomal recessive connective tissue disorder characterized by tortuosity and elongation of the large and medium-sized arteries and a propensity towards aneurysm formation and vascular dissection. ATS is caused by mutations in *SLC2A10* encoding the facilitative glucose transporter 10 (GLUT10), whose role in the ATS pathogenesis remains still controversial. We recently showed that GLUT10 deficiency causes the dysregulation of several genes/proteins involved in TGFβ signaling, extracellular matrix architecture and pathways that control oxidative stress response. GLUT10 should be located intracellularly; however, neither the exact localization, i.e., nuclear membrane, mitochondria, or endoplasmic reticulum (ER), nor the transported substances, i.e., glucose or dehydroascorbic acid (DAA), have been demonstrated.

Here, we demonstrate that GLUT10 facilitates DAA uptake into the endomembranes and, in particular, into ER. GLUT10 produced by *in vitro* translation and incorporated into proteoliposomes efficiently transports DAA. Silencing of GLUT10 in hTERT immortalized human fibroblasts compromised DAA transport activity through the endomembranes. Similarly, in plasma membrane-permeabilized ATS fibroblasts a huge decrease in DAA transport was observed and the stable re-expression of GLUT10 restored the impaired DAA transport activity. Immunocytochemistry of human control fibroblasts showed a perinuclear abundance of GLUT10. Immunoblotting of subcellular fractions from human control fibroblasts revealed that GLUT10 was principally present in the microsomal fraction, containing ER-derived vesicles, as showed by the presence of the specific ER marker proteins GRP78 and GRP94, and by the almost complete absence of mitochondrial and cytoplasmic markers, VDAC1, cyclophilin D, and GAPDH, respectively. Transient expression of V5-tagged GLUT10 in ATS patients' fibroblasts and co-localization experiments with the specific ER marker PDI definitely confirmed the ER localization of GLUT10.

Overall, the present findings demonstrate that GLUT10 facilitates DAA uptake into the ER lumen and likely to the nucleoplasm through the nuclear envelope, which is a subdomain of the ER. Our findings support both "antioxidant-" and "enzyme cofactor-" models of a vitamin C-related pathology. Indeed, AA acts as an antioxidant/electron acceptor protecting against oxidative stress-induced cellular damage by scavenging free radicals also during the process of oxidative protein folding. Furthermore, AA is an essential cofactor for α-ketoglutarate-dependent dioxygenases, such as prolyl and lysyl hydroxylases inside the ER and for ten-eleven translocation demethylases and the Jumonji protein family present in the nucleus. Thus, shortage of AA in the lumenal compartments of the secretory pathway and in the nucleoplasm can depress the production of extracellular matrix proteins at both post-translational and epigenetic levels.