Epigenetic effects of ascorbate in the pathomechanism of scurvy and arterial tortuosity disease

(final report)

Ascorbate has been recently shown to have a role in epigenetic regulation as a cofactor of Fe^{2+}/2-oxoglutarate dependent dioxygenases. The members of this enzyme family can demethylate histones and DNA, affecting gene expression. These findings suggest that the intracellular and especially the nuclear ascorbate concentrations can have an epigenetic role. We supposed that GLUT10 localizes in the nuclear envelope and mediates dehydroascorbic acid (DAA) transport into the nucleoplasm. Fibroblasts from arterial tortuosity syndrome (ATS) patients were used as a model for local (nuclear) hypovitaminosis C. Loss-of-function mutations in the *SLC2A10* gene encoding GLUT10 are responsible for this rare connective tissue disorder. GLUT10 belongs to a family of transporters that catalyze the uptake of sugars/polyols by facilitated diffusion.

Since intracellular distribution of the transporter was dubious, first we aimed to clarify the subcellular localization of GLUT10. In silico GLUT10 localization prediction suggested its presence in the endoplasmic reticulum (ER). Immunoblotting showed the presence of GLUT10 protein in the microsomal, but not in mitochondrial fractions of human fibroblasts and liver tissue. An even cytosolic distribution with an intense perinuclear decoration of GLUT10 was demonstrated by immunofluorescence in human fibroblasts, whilst mitochondrial markers revealed a fully different decoration pattern. GLUT10 decoration was fully absent in fibroblasts from three ATS patients. Expression of exogenous, tagged GLUT10 in fibroblasts from an ATS patient revealed a strict co-localization with the ER marker protein disulfide isomerase (PDI). The results demonstrate that GLUT10 is present in the ER, including the nuclear envelope [1].

In the forthcoming study GLUT10-mediated DAA transport was investigated, supposing its involvement in the pathomechanism. GLUT10 protein produced by in vitro translation and incorporated into liposomes efficiently transported DAA. Silencing of GLUT10 decreased DAA transport in immortalized human fibroblasts whose plasma membrane was selectively permeabilized. Similarly, the transport of

DAA through endomembranes was markedly reduced in fibroblasts from ATS patients. Re-expression of GLUT10 in patients' fibroblasts restored DAA transport activity. The results demonstrated that GLUT10 is a DAA transporter and DAA transport is diminished in the endomembranes of fibroblasts from ATS patients [2].

Since the role of GLUT10 as a DAA transporter of endomembranes was verified, we moved towards the investigation of epigenetic effects. We observed an ascorbate accumulation upon ascorbate addition in control fibroblasts, which was diminished in cells from ATS patients. Increased cytosine methylation and decreased hydroxy-methylation was found in the DNA of ATS fibroblasts. Ascorbate addition could reverse these changes in control cells only. Similar changes were observed in the gene of PPAR-gamma, a transcription factor has a crucial role in the regulation of synthesis of extracellular matrix proteins. The manuscript containing these data will be submitted within this year.

In a review we overviewed the specific functions of ascorbate, which are compartmentalized within the eukaryotic cell. The review partially predicted the results published in the two original papers mentioned above. The review focused on the reactions and transporters that can modulate ascorbate concentration and redox state in three compartments: endoplasmic reticulum, mitochondria and nucleus. By introducing the relevant experimental and clinical findings we made an attempt to coin the term of ascorbate compartmentation disease [3].

During the research period we published two reviews on the redox homeostasis of the ER, with special emphasis on two prooxidants known to be generated in this compartment and influence local ascorbate level: hydrogen peroxide and 4-hydroxynonenal [4,5].

The research reported here was executed in the frame of an international cooperation with the lab of Marina Colombi (Brescia University), in collaboration of the team of Paul J. Coucke (Ghent University) and Angelo Benedetti (Siena University).

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