Report on the Doctoral Dissertation of Dr Christos Chinopoulos titled: Mitochondrial substrate-level phosphorylation: from salvaging hypoxic cells to a promising cancer target, submitted to the Hungarian Academy of Sciences

The dissertation matches to the formal requirements of the Hungarian Academy of Sciences, summarizing the scientific contribution of the author/candidate over the last decade. It represents a high quality continuation of the work, started in the research school initiated by professors Veronika Ádám and László Tretter, based in the Biochemistry Department of the Semmelweis University Budapest. The candidate got the oppportunity to participate in the actitivities of additional internationally recognized research centers, making his scope broader, and extended the available tachniques. The research results are published in 33 peer reviewed papers, with a significant number of citations by the top scientists of the field. I see this as an evident indication of the impact and the merit of the authors contributions toward the better undersanding of the field studied.

The dissertation is focussed around two basic scientific questions. The first (and more delightful for the opponent) part is a sound basic study on the mitochndrial transport systems, the involvement of the main metabolic processes in the regulation of the citric acid cycle and the energy household of the cells, with special focus on the Adenine Nucleotide Tarnsporter as well as the ATP sythesis both by the F1F0 ATPase/ATP synthase and the substare level phosporylation. The second part is a thorough analysis of the variability of certain citric acid cycle isoenzymes in certain nervous tissue cell types, and their possible involvement in hypoxic adaptation /survival as well in tumor genesis and progression processes. The two areas are linked and substantiated with sufficient previous results, and proper interpretation, making the dissertation a comprehensive piece of contribution toward the deeper understanding of mitochondrial biochemistry and pathobiochemistry indeed. The literature cited is numerous, updated and relevant, making clear the position of the candidates work in the field.

New results of the candidate

He studied and characterized the kinetic features of the Adenine Nucletide Transporter (ANT). Developed a new analytical techniques to follow ATP, ADP transport, transmembrane electrochemical potential gradient. The reversibility of the ANT transporter allows the transport to turn backward, making mitochondria a net ATP consumer instead of energizing the whole cell through Oxidative Phospohorylation. Substrate level mitochondrial ATP synthesis may serve and

contribute as an alternative to the protection of mitochondrial ATP consumption form the cytosol in metabolic collapses as well as under hypoxic conditions.

Working with isolated mitochondria as a model system allows the maintenance of well defined electrochemical conditions and chemical concentration features, although the interpretation of the data obtained, is sometimes difficult to transfer in terms of in situ working conditions for the cell organelle, since modelling the chemical- electrochemical features, particularly outside are rather arbitrary, genarting artefacts sometimes. The candidate made progress in this area with applying his analytical method in permeabilized cells and tissue homogenates as well. His kinetic assay method turned into a standard piece of recommeneded techniques in mitochondrial research handbooks.

Question 1. The sophisticated transport model developed and used by the author does not include the impact of Calcium ion trafficking, meanwhile their uptake and impact, both on the transmembrane potential gradient, and as a competitor for the inorganic Phoshate, available at the matrix side is documented and discussed in basic pathobiochemistry textbook since long. What was the reason not to include the traffic of the divalent kation Calcium into the rather comprehensive calculation model? How the model would behave under conditions when Calcium acculumation in the matrix space occurs?

Question 2. Brown adipose tissue mitochondria are unique in their controlled uncoupling phenomena through Thermogenin channel proteins. Is there any evidence about the mitochondrial Adenine Nucleotide Transporter function in these tissues? Is there any relevance of ANT systems and mitochondrial substarete level Phosphorylation in the thermoregulation of the body? Does it have any possible involvement in body weight control with UCP protein expressions in human white adipose tissue?

In the second part of the dissertation a thoroughful immunochemical and metabolic analysis is presented about the hetrogeneity of the citric acid cycle and other mitochondrial main metabolic pathway enzymes among different cell types. The convincing results indicate the possible involvement of the substare level phosphorylation enzyme complex isoforms in the maintenance of the necessary ATP level under anaerobic, energy deficient conditions to promote cell survival. However tehir activaton may contribute to the growth and propagation of certain tumor cell types as well. Interference with these enzyme activities may contribute to the tumor therapy in particular nervous tissue cell types.

Question 3. How the major differences in mitochondrial enzyme/isoenzyme expression profiles among different brain cell types would fit to the endosymbiotic model of the mitochondrial origin? Which signalling pathways could be involved in the cell type specific differentiation of mitochondrial enzyme profiles and transporter systems?

Question 4. Heteroplasmic phenomena may alter the metabolic features in the frequently dividing tumour tissues. Does it have any positive or negative impact on the tumor progression process?

Question 5. Are there any new data on the possible application of the substrate level mitochondrial phosphorylation system manipulations as a tool in tumor therapy, as promised in the dissertation?

Summarizing the opinion of the opponent: I am convinced about the scientific merit of the candidate and his contribution toward a better understanding of a basic biochemical question and its medical application. The published results are sound, supported by a broad set of experimental data, obtained through state of the art methods. A significant part of these methods were developed and established by the candidate himself. The interpretation of the new data is well founded, moderate and properly integrated into the existing common knowledge on the field.

I recommend the dissertation for a public defense and discussion process.

Szeged, 2023. 01. 09.

László Dux MD PhD DSc
Professor of Biochemistry and
Clinical Pathology