Mitochondrial Permeability Transition Pores

Subjects: Pathology

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Substantial evidence has revealed that mitochondrial permeability transition pores (mPTPs) are associated with signaling pathway of cardioprotective models and seem to be an end-effector of cardioprotection. Experimental streptozotocin-induced diabetes mellitus (D) was shown to provide sufficient protection to the myocardium via compensatory mechanisms enabling mitochondria to produce energy under pathological conditions during the acute phase.

The hypothesized involvement of mPTPs in these processes prompted us to use liquid chromatography and mass spectrometry-based proteomic analysis to investigate the effects of the acute-phase D condition on the structural and regulatory components of this multienzyme complex and the changes caused by compensation events.

We detected ADT1, ATP5H, ATPA, and ATPB as the most abundant mPTP proteins. The between-group differences in protein abundance of the mPTP complex as a whole were significantly upregulated in the D group when compared with the control (C) group (p = 0.0106), but fold changes in individual protein expression levels were not significantly altered except for ATP5H, ATP5J, and KCRS. However, none of them passed the criterion of a 1.5-fold change in differential expression for biologically meaningful change. Visualization of the (dis-)similarity between the C and D groups and pairwise correlations revealed different patterns of protein interactions under the C and D conditions which may be linked to endogenous protective processes, of which beneficial effects on myocardial function were previously confirmed.

Our results point to the involvement of mPTP proteins in the endogenous protective processes leading to the preservation of myocardial function under pathological conditions. Proteomic studies with respect to the correlation of mPTP proteins were shown to be one of the most promising options for the advancement of mPTP regulation mechanisms. Subtle changes in mPTP protein expressions, as well as mutual relationships between proteins, may be sufficient to contribute to preserving mitochondrial energy metabolism under the increased energy load represented by experimental D.

mitochondrial permeability transition pores | cardioprotection | experimental diabetes mellitus | proteomic analysis | endogenous protective processes | heart | mitochondria | adaptation | mitochondrial signalling

1. Introduction

Mitochondrial permeability transition pores (mPTPs) are a nonspecific, highly conductive channel enabling passage of molecules smaller than 1.5 kDa through the inner mitochondrial membrane (IMM). The pores are primarily

formed and opened upon an increase in Ca^{2+} in the mitochondrial matrix, signaling by reactive oxygen species (ROS) or inorganic phosphates, or intracellular acidification [1][2][3][4][5].

mPTPs are unique not only due to their multifunctional nature, but also due to their complex dynamic structures. Unlike typical membrane pores, the structure and composition of mPTPs constantly change with respect to the cell's actual requirements [3][6][7]. The exact molecular identity of mPTP components remains subject to debate and investigation among researchers [1][6].

The first references to the existence of mPTPs date back to the 1970s, with several hypotheses about their structure being proposed since. However, the molecular identity of mPTPs remains unclear. Every year, new knowledge about the structure and regulation of this mitochondrial pore appears.

The mPTP is probably a multiprotein complex containing individual proteins with structural or regulatory functions [3][8][9]. An in-depth analysis of these protein–protein interactions was assumed to help uncover a potential mechanism of mPTP regulation. The components falling within the current concept of the mPTP molecular assembly include, besides adenosine triphosphate (ATP) synthase and its subunits [10], adenine nucleotide translocator (ADT). New findings introduced by [11] confirmed the participation of ADT in the mPTP structure. Other mPTP components, namely, voltage-dependent anion channel (VDAC), cyclophilin D (CypD) [12], and a phosphate carrier protein (MPCP) [13], were considered to be involved in the regulation of mPTP activity [1][14][15]. Most proteins that regulate mPTPs directly or indirectly bind to ATP synthase and/or to CypD [16].

Among other regulatory components, members of the Bcl-2 family of proteins and translocator protein (TSPO) were also found, and recently, the involvement of hexokinase-2 (HK-2) and mitochondrial creatine kinase (mtCK) proteins in mPTP regulation was demonstrated [3][8]. The structural role of HK-2 and mtCK consists in stabilization of the contact sites between the inner and outer mitochondrial membranes (IMM and OMM, respectively) through interactions with ADT and VDAC [4]. The cardioprotective effects of mtCK consist in increasing the amount of functional mtCK octamers [17]. Similar protective effects were reported following inhibition of HK-2 dissociation, thereby preventing opening of mPTPs [18]. Understanding the structure–function relationships and regulation of mPTPs is crucial for the development of cardioprotective strategies [19].

At present, mPTP regulation is mostly observed at the level of single changes in single structural or regulatory proteins. So far, however, there has been no consistent information concerning possible interactions between proteins involved in mPTP regulation, which would eventually lead to the protection of the heart from injury.

The aim of the present study was therefore to evaluate whether the cardioprotection conferred by an experimental model of acute D influenced mPTP composition. Since mPTPs form a multienzyme complex, proteomic analysis was considered to be a suitable tool for their characterization.

Functional insight most often requires quantitative comparison between two or more biological states. To fully understand the regulation of protein spectra under both conditions, changes in protein abundances should be

analyzed in more detail at the molecular level. While liquid chromatography and mass spectrometry (LC-MS) is not inherently quantitative, this limitation was successfully overcome by the introduction of stable isotopes into the molecules to be identified, or alternatively, by label-free approaches.

We chose LC-MS-based proteomics to investigate a model of experimental, streptozotocin-induced diabetes mellitus (which is referred to as “experimental D” throughout the text), with the aim of clarifying the cardioprotective function of cardiac mitochondria at the level of mPTP regulation. Experimental D in the acute stage exhibits all signs of diabetes in the sense that the observed disruption/disorganization of the main metabolic pathways can only be attributed to acute D, as it is not confounded by complications developing due to an ongoing diabetic condition [20][21][22]. For this reason, all adaptation changes developing in the myocardium in the period of acute D are devoid of all the interfering comorbidities incurred by the chronic D condition, and thus can be investigated [23]. The endogenous protective mechanisms present in the acute phase of experimental D were shown to maintain cardiac function at the subcellular level in a similar way to ischemic preconditioning and remote ischemic preconditioning. Therefore, acute-phase experimental D was proven to be a suitable model for the study of endogenous protective mechanisms and can be thought of as a type of metabolic preconditioning [24][25][26].

The experimental model of D also appears to be well-suited for monitoring possible regulations at the mPTP level. The model described herein involves D-induced damage manifested by an enhanced calcium transient, an increased myocardial energy load, and respiratory chain dysfunction. The progressively accelerated rate of glycolysis in acute-phase D gradually exhausts the supply of oxidized cofactors, resulting in a pseudohypoxia condition when the respiratory chain, despite having enough oxygen, cannot adequately utilize it. The resulting anaerobic glycolysis further exhausts the supply of oxidized cofactors. Hence, the condition of pseudohypoxia produces an excess of lactate, causing the ratio of lactate to pyruvate to elevate, while the ratio of oxidized and reduced form of nicotinamide adenine dinucleotide (NAD+/NADH) falls. On the other hand, this condition also triggers endogenous protective mechanisms to compensate for the damage caused by the noxa, ultimately leading to maintenance of the energy and dynamic balance of the system and to better survival of the acute diabetic myocardium subjected to overload [21][27][28].

2. Conclusion

Based on the available knowledge, the identification of protein–protein interaction networks and functional interconnection of mPTP-forming and mPTP-regulating proteins may provide interesting insights into the way mPTPs are involved in the cardioprotection process. Furthermore, we aimed to clarify whether cardiac mitochondria could maintain mPTP protein expression at the level of healthy cardiac mitochondria under experimentail D conditions.

The regulation of the opening and closing of mPTPs is the subject of research by several teams dealing with cardioprotection, but the question of whether regulation is associated with changes in expressions and interactions at the level of individual protein components of the multienzyme mPTP complex, keeping the pores in the closed state, is still unresolved. Using the described experimental model of D, we attempted to determine which changes

in the mPTP protein expression could be identified as being associated with the condition that was already proven to trigger adaptation to pathological stimuli. Ultimately, our aim was to find out how these changes contributed to cardioprotective mechanisms that maintain the dynamic equilibrium of the system and ultimately lead to preserving mitochondrial energy metabolism under load conditions represented by acute diabetes mellitus.

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