Proteomic and Metabolomic Studies of Cardiac Metabolism in PKCδ null mice

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Introduction: Protein Kinase C (PKC) is a heterogeneous family of phospholipid-dependent kinases. PKCδ is among the predominant isoforms of PKC in cardiac ventricles and is believed to play an essential role in cardiomyocyte growth. It has been implicated in heart failure, myocardial hypertrophy and ischemic preconditioning. Ischemic preconditioning confers cardiac protection during subsequent ischemia-reperfusion.

Aims: In the present study, proteomic and metabolomic analysis was used to investigate the effect of PKCδ on cardiac metabolism using the recently created first PKCδ-knockout mouse model [1] and to determine whether the protein changes can be correlated to the metabolic changes.

Methods: *Animal model:* PKCδ-deficient (PKCδ-/-) mice were generated by targeted disruption of an endogenous PKCδ-/- gene [1]. Hearts were harvested for proteomic and metabolomic studies from a group of PKCδ-/- and wild-type (PKCδ+/+) adult mice. *Proteomic analysis:* Protein extracts of murine hearts were separated by two-dimensional gel electrophoresis and proteins were identified by Matrix Absorption Laser Dissociation Ionisation Mass Spectrometry (MALDI-MS). *Metabolomic analysis:* ^{1}H high-resolution NMR was used to analyse the acid extract of the mouse hearts. NMR spectra were obtained using a 500MHz Bruker spectrometer and sodium 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate was added to the samples for chemical shift calibration and quantitation.

Results: Proteomic analysis of the heart protein extracts revealed profound changes in enzymes related to energy metabolism in PKCδ-/- hearts. Some isoforms of glycolytic enzymes, e.g. lactate dehydrogenase and pyruvate kinase were absent or decreased, while several enzymes involved in lipid metabolism, e.g., acyl-CoA dehydrogenases, showed a marked increase in PKCδ-/- hearts when compared with controls. Consistent with this, high resolution NMR of the PKCδ-/- heart extracts showed a significant decrease in the ratio of glycolytic end products (alanine+lactate) to end products of fatty acid metabolism (acetate+β-hydroxybutyrate) when compared to wild-type controls (Figure 1). A summary of the NMR observed metabolites in PKCδ-/- and PKCδ+/+ heart extracts is shown in Table 1.

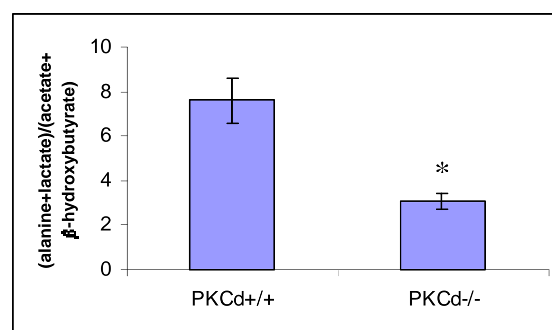

Figure 1: The ratio of (alaine+lactate)/(acetate+β-hydrobutyrate) in PKCδ-/- and PKCδ+/+ hearts. Data expressed as mean +/- s.e.m. * P < 0.04 when compared with controls.

Table 1: Metabolites in PKCδ-/- and PKCδ+/+ heart extracts observed by NMR:

	PKCδ-/-	PKCδ+/+		PKCδ-/-	PKCδ+/+
Alanine	1.25 ± 0.14	1.00 ± 0.22	Lactate	6.32 ± 0.99	5.80 ± 0.99
Creatine	5.58 ± 0.19	5.16 ± 0.56	Acetate	0.57 ± 0.09	1.00 ± 0.33
Choline	0.17 ± 0.06	0.22 ± 0.12	Succinate	0.40 ± 0.06	0.41 ± 0.10
Carnitine	0.45 ± 0.05	0.41 ± 0.09	β-hydroxybutyrate	0.43 ± 0.13	1.37 ± 0.70
Taurine	13.89 ± 0.70	14.01 ± 2.23	Glycine	0.22 ± 0.01	0.23 ± 0.03
Glutamate	1.13 ± 0.21	1.31 ± 0.38	Phosphocholine	0.11 ± 0.01	0.12 ± 0.01

Data are expressed as mean +/- s.e.m. N = 3.

Discussion: Combining the information obtained from the proteomic and metabolomic studies, our data showed that the loss of PKCδ caused a metabolic shift from glycolytic to lipid metabolism in murine hearts. The proteomic alterations in the PKCδ-/- hearts were supported by the metabolic changes. Therefore, metabolomic and proteomic studies could be complementary in elucidating gene functions following gene manipulations.

[1] Leitges M et al. J Clin Invest (2001);108: 1505-1512.