Localization and Partners of polycystin 1 at MAMs and Mitochondria

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INTRODUCTION

In line with growing evidence demonstrating a role for alterations in cell metabolism in ADPKD, we have recently shown that the polycystins modulate mitochondrial function by regulating mitochondrial calcium uptake. Interestingly, the polycystins localize at mitochondria-associated membranes (MAMs), regions of close apposition between the endoplasmic reticulum (ER) and mitochondria, considered to be hotspots for calcium transfer. In order to better understand the relationship between the polycystins and mitochondria, we performed a mass spectrometry screening to identify *in vivo* interactors of PC1 at MAMs.

METHODS

Crude mitochondria (mitochondria and MAMs), isolated from P20 Pkd1-BAC mice expressing HA-tagged PC1, were incubated with a non-permeable cross linker followed by lysis in PBS + 1% SDS. The samples were then diluted 10 times in SDS-free lysis buffer, immunoprecipitated using magnetic HA-beads, and the eluate sent for mass spectrometry. In addition, PC1-CTT constructs with N-, C-terminal, or no HA tag were transfected in HEK293 cells, and their localization was analyzed by confocal microscopy.

RESULTS

Our analysis identified mitochondrial membrane proteins as well as mitochondrial matrix proteins. This finding is particularly interesting, as the small C-terminal tail (CTT) of PC1, which has been demonstrated to translocate into the nucleus by our group and others, has recently been shown to localize within mitochondria. In an effort to further investigate the localization of PC1-CTT, we generated PC1-CTT constructs with N- or C-terminal tags, as well as without any tag, and expressed them in HEK293 cells. Surprisingly, we found that tag positioning affects the subcellular localization of PC1-CTT. Moreover, the no-tag construct shows both mitochondrial and nuclear localization, suggesting the interesting possibility that the PC1-CTT can exhibit different localizations depending on as yet unknown factors.

CONCLUSION

Our study identified novel interactors of PC1 both at MAMs and mitochondria, and indicated that PC1-CTT could act at two different levels in the cell, in the nucleus, where it regulates gene transcription, and in mitochondria, where it might affect metabolism.