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Neuronal p60TRP expression modulates cardiac capacity

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ABSTRACT

Heart failure, including myocardial infarction, is the leading cause for death and the incidence of cardiovascular diseases is predicted to continue to rise worldwide. In the present study we investigated the whole heart proteome profile of transgenic p60-Transcription Regulator Protein (p60TRP) mice to gain an insight into the molecular events caused by the long-term effect of neural p60TRP over-expression on cardiac proteome changes and its potential implication for cardiovascular functions. Using an iTRAQ (isobaric tags for relative and absolute quantitation)-based proteomics research approach, we identified 1148 proteins, out of which 116 were found to be significantly altered in the heart of neural transgenic p60TRP mice. Based on the observed data, we conclude that *in vivo* neural over-expression of transgenic p60TRP with its neuroprotective therapeutic potential significantly affects cardiovascular capacities.

1. Introduction

P60TRP is a member of the G-protein-coupled receptor associated sorting protein (GPRASP) family. In recent years, extensive research has revealed numerous interacting partners of G protein-coupled receptors (GPCRs) and one of which is the GPRASP family [1,2]. P60TRP is also known as GASP3 or BHLHB9 [3,4] and among the many distinguishing features of p60TRP it is noteworthy that it contains a myc-type basic helix–loop–helix (bHLH) domain at its C-terminus which is a protein structural motif that characterizes transcription factors. Our studies have shown that p60TRP localized both in the cytoplasm and the nucleus of cells and has been predominately expressed in the nervous system, the heart and skeletal muscle [3,4]. Recently, we have reported a newly established transgenic p60TRP mice revealing its neuroprotective mode of operation within the central nervous system (CNS) [4].

Recent advances in proteomics technologies offer opportunities to study the entire proteome of any sample in a single experiment. In comparison with previous gel-based methodologies, the two-dimensional (2D) liquid chromatography coupled with tandem mass spectrometry (2D-LC–MS/MS)-based multidimensional protein identification technology [5] combined with multiplex isobaric tag for relative and absolute quantification (iTRAQ) [6] provides an alternative approach for quantitative proteomics profiling. This sensitive technique allows the simultaneous quantification of proteins in four-plex samples [7]. Recently, we have successfully applied the high-throughput iTRAQ-LC–MS/MS strategy in the area of neuro-degenerative diseases [8,9]. Our p60TRP transgenic mice consistently revealed higher heart volumes compared to wild-type littermates indicating a pivotal regulatory role of p60TRP on neuromuscular junctions in maintaining cardiac output. Accordingly, we applied the iTRAQ-based proteomics to our clinically relevant transgenic mouse model for

Abbreviations: p60TRP, p60 Transcription Regulator Protein; iTRAQ, isobaric tags for relative and absolute quantitation.

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quantitative profiling of p60TRP-regulated cardiac genes. Subsequently, the iTRAQ-based proteomics–bioinformatics platform was used to generate the proteome comprising p60TRP regulated proteins from the transgenic mice-derived heart. Finally, the altered expression of some of the regulated proteins was validated by western blot analyses to delve into their cardiac activities (Fig. 1).

Since p60TRP is a novel protein with many unidentified features, our present investigations could further contribute to its operational assignment by providing substantial information regarding its functional influence on neuromuscular junctions and related myocardial diseases. Our *in vivo* results revealed the long-term effect of neural p60TRP over-expression on cardiac proteome changes and its potential implication for cardiovascular functions.

2. Materials and methods

2.1. Reagents

Unless indicated, all reagents used for biochemical methods were purchased from Sigma-Aldrich (St. Louis, MO, USA). Materials and reagents for SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) were from Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA). The iTRAQ reagent

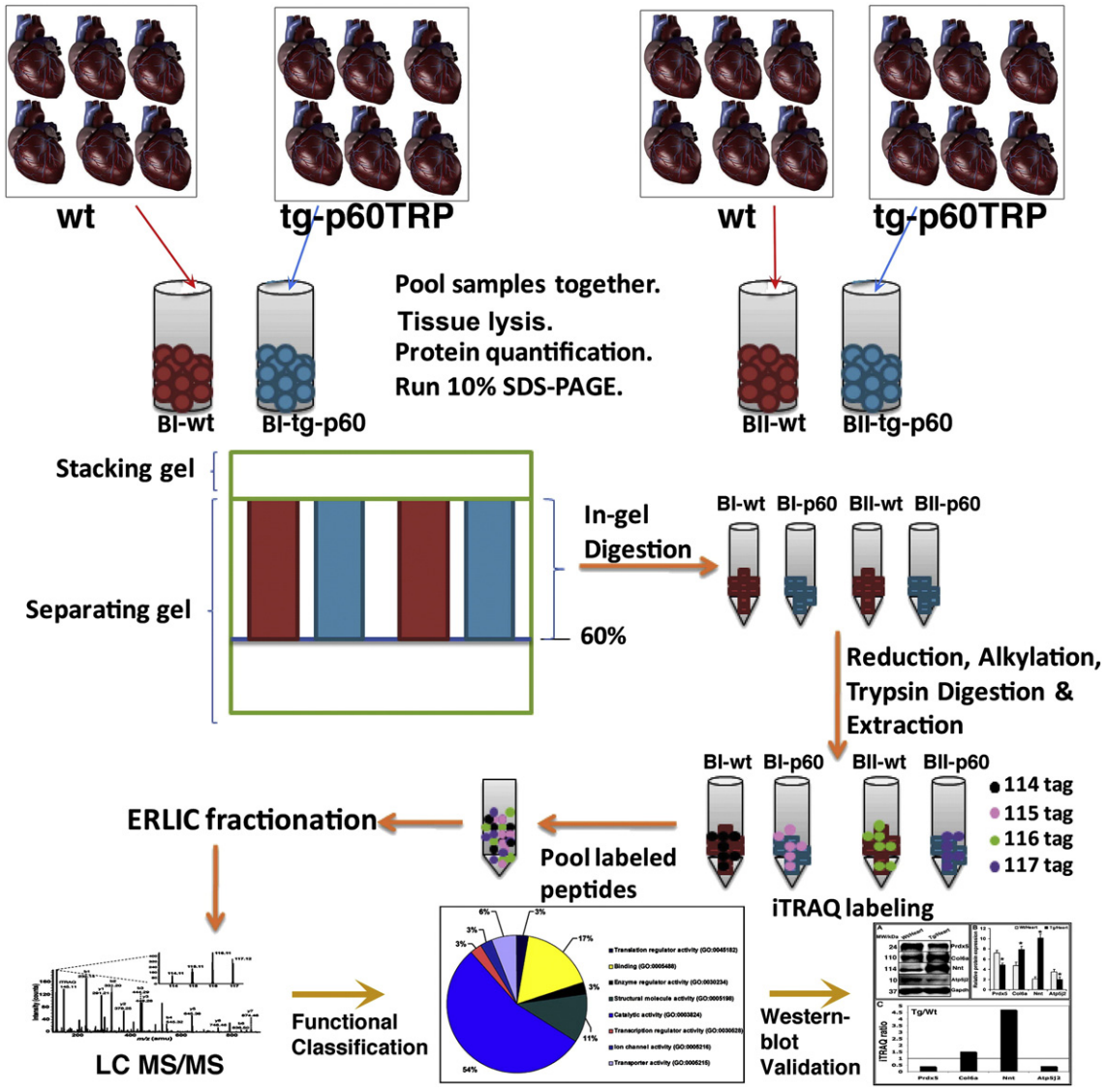

Fig. 1 – Schematic representation of the experimental design showing biological and technical replicates. Following heart-derived tissue lysis, protein extracts were acetone precipitated and quantified, ran in SDS-PAGE and subsequently digested. The quantitative proteomics analyses of transgenic p60TRP mice-derived hearts was performed by labeling with multi-plex isobaric tags (114, 115, 116 and 117) for relative and absolute quantification (iTRAQ) reagent followed by Electrostatic Repulsion–Hydrophilic Interaction Chromatography (ERLIC)-based fractionation, and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS)-based multidimensional protein identification technology. The obtained data was analyzed using ProteinPilot software and validated by quantitative western blots. Finally, proteins were functionally classified into various subgroups.