Application of SILAC-based mass spectrometry for determining the phosphoproteome in continuously or interval-paced HL-1 cardiomyocytes

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Purpose:
Atrial fibrillation (AF), the most common arrhythmia in clinical practice, is characterized by electrical, contractile and structural remodeling of the atria. Many of the adverse atrial remodeling processes during AF are associated with or caused by reversible alterations of protein-phosphorylation. However, aberrant cellular signaling in AF remains to be elucidated fully. Here, we report the systematic characterization of AF-dependent alterations in signal transduction / phosphoproteome in a model of AF using the murine atrial cardiomyocyte cell line HL-1.

Methods: HL-1 cells were subjected to (i) continuous and (ii) interval rapid pacing (RAP) (Fig. 1). The phosphoproteomes of RAP HL-1 cells were analyzed in the presence of an internal SILAC-standard by phosphopeptide enrichment and high-accuracy mass spectrometry (HPLC-MS/MS). Altered protein phosphorylation at both tyrosine and serine/threonine phosphorylation sites was identified and quantified by analyzing the meta-data using bioinformatic approaches.

Results: For the first time, the phosphoproteomes of HL-1 cells exposed to 24 hours continuous or interval RAP, respectively, were systematically determined. RAP-dependent changes were observed in response to both continuous and interval-pacing. While there was a considerable overlap of phosphoproteins equally affected under both RAP conditions, fairly selective alterations could be assigned to the individual groups as well. Phosphoproteins that were subject to RAP-dependent alterations were categorized according to protein families, signaling pathways, and cellular functions.

Conclusions: Divergent phosphorylation patterns as observed in response to either continuous or interval RAP supports the view that atrial remodeling due to short episodes of RAP or AF could be attenuated by existing recovery mechanisms. These may help to limit AF progression and add to our basic understanding of mechanisms underlying reversibility of early atrial remodeling during AF.