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EDITORIAL COMMENT

Affinity Proteomics for Phosphatase Interactions in Atrial Fibrillation*

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Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice, with an estimated 30 million individuals affected worldwide (1). AF constitutes a major risk factor for stroke and heart failure, resulting in significant morbidity and mortality. Remodeling of the atria is a consequence as well as a substrate for perpetuation of AF. The remodeling process occurs at various levels, including atrial fibrosis, cardiomyocyte contractibility, and electrical coupling. During electrical remodeling, an unbalanced calcium (Ca^{2+}) transport exposes cardiomyocytes locally to increased Ca^{2+} levels. Ca^{2+} overload is thought to trigger the activation of Ca^{2+}-dependent calcineurin and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which is involved in electrical remodeling and the initiation of cell death pathways (2). Initially, Ca^{2+}-dependent activation leads to autophosphorylation of CaMKII, which in turn induces sustained Ca^{2+}-independent activation of this kinase. Later, reactive oxygen species contribute to Ca^{2+}-independent CaMKII activation via oxidation of methionines (3). Inhibition of CaMKII showed benefits in animal models of AF (4,5), but most ion channels and enzymes involved in Ca^{2+} handling are ubiquitously expressed (6), and no antiarrhythmic drug currently targets atria in a cell-type-specific manner.

Regulatory subunits of phosphatases may represent an alternative target. Interestingly, protein phosphatase 1 (PP1) and 2 (PP2A), together with calcineurin, constitute almost 90% of the total protein phosphatase activity in the heart (7). Protein phosphatase holoenzymes comprise multimeric enzymes with their regulatory (R) rather than catalytic (PP1c) subunits being responsible for subcellular localization, substrate specificity, and activity. PP1c forms complexes with more than 50 regulatory subunits. More than one-half of the human proteins undergo reversible phosphorylation (8). Thus, a comprehensive characterization of phosphatase-holoenzyme complexes in human cardiac tissue is required.

SEE PAGE 163

In this issue of the *Journal*, using affinity proteomics (**Figure 1**), Chiang et al. (9) investigated interaction partners of PP1c in AF. Coimmunoprecipitation experiments were performed in right atrial specimens from patients as well as in cardiac tissue from mice. PP1c interaction partners were identified in human and mouse cardiac tissue. In human, only 9 interaction partners had previously been designated as putative R-subunits (PP1-interacting holoenzymes) in atrial tissue. The authors acknowledged that abundant proteins can be contaminants and identified regardless of their interaction specificity in coimmunoprecipitation experiments (10). They substantiated the specificity of the binding partners by using a bioinformatic screen: 60 of the 78 proteins immunoprecipitated from human atria contained at least 1 of 3 known PP1c-docking motifs (namely, RVxF, MyPhoNE, and SILK). Similar results were obtained in mice. Next, the authors (9) quantified the relative binding of the known R-subunits to PP1c. Label-free quantification by mass spectrometry offers an estimate of protein abundance because the number of analyzable peptides is proportional to the sequence length of the respective protein. Nonetheless, the estimate varies depending on the amino acid sequence and the ionization efficiency of the digested

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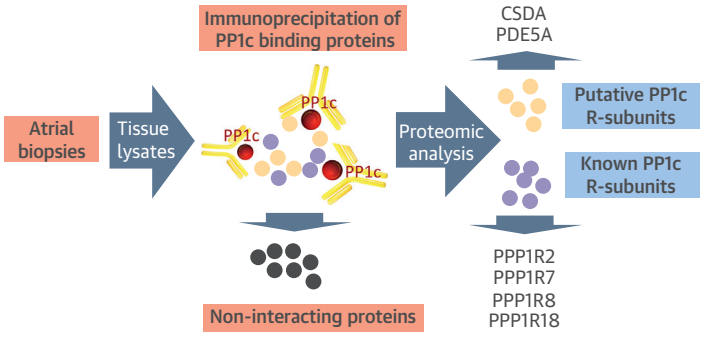
peptides. Low-abundance proteins are usually less reliably quantified by proteomics. Known R-subunits showed the highest relative binding to PP1c: PPP1R7 and PPP1R2 were among the top interactors in mice and human, whereas PPP1R8 and PPP1R18 showed more binding in human and PPP1R11 was only found in mice.

For 3 putative candidates, their interaction with PP1c was further validated: protein transport Sec31A (SEC31A), valosin-containing protein (VCP), and cold-shock domain protein A (CSDA) were coimmunoprecititated with PP1c in HEK293 cells. Then, the authors (9) explored differences in the PP1c interactome between patients with sinus rhythm and those with paroxysmal AF. PPP1R7, CSDA, and PDE5A had increased binding to PP1c in patients with paroxysmal AF. Stronger binding, at least for 2 of the candidate targets, was observed in the absence of increased expression of the R-subunits or PP1c, suggesting that conformational changes in the PP1c complex might contribute to the alterations in phosphorylation levels in AF. CSDA is a transcriptional factor and represses the activity of hypoxia inducible factor-1α and nuclear factor κB in the nucleus of HeLa cells (11). CSDA localizes to the sarcolemma of isolated cardiomyocytes. The authors (9) suggest potential roles for CSDA in AF. PDE5A is a cyclic guanosine 3′,5′-monophosphate-dependent-specific phosphodiesterase. Inhibition of PDE5A prevents cardiac hypertrophy and left ventricular dysfunction associated with oxidative stress and extracellular matrix production in mice (12). Cardiac fibroblast proliferation and extracellular matrix secretion contribute to Ca^{2+} re-entry pathways in AF (13).

Proteomics has been used for interrogating cardiac metabolism (14), characterizing cardiac fibrosis (15), or exploring changes in myofilament composition (16,17). Affinity proteomics opens new avenues for studying molecular interactions. Coimmunoprecipitation, however, can be notoriously prone to unspecific binding (10). Moreover, experiments in tissue lysates may not accurately reflect the in vivo interactions. PP1c is expressed in many cell types, including cardiomyocytes and cardiac fibroblasts. Tissue lysates will contain R-subunits of different cell types, which would be spatially separated in intact tissue. Also, age and sex could be possible confounders in this analysis: patients with paroxysmal AF were on average 10 years older and predominantly female, whereas patients in sinus rhythm were almost entirely male. Finally, the study was performed in right atrial tissue. In the context of AF, it would be interesting to validate these findings in left atria. Thus, further studies are required to understand how the putative R-subunits alter PP1c activity and whether they could pave the way for novel interventions to control phosphorylation levels in AF.

FIGURE 1 Affinity Proteomics Workflow



Known and putative regulatory subunits were identified based on their interaction with the catalytic subunit of protein phosphatase 1 (PP1c). CSDA = cold-shock domain protein A; PDE5A = phosphodiesterase type-5A; PPP1R = PP1 regulatory subunit.

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