

## Reversal of Hypertrophy-dependent Retention of Calsequestrin in Endoplasmic Reticulum using Protein Kinase CK2 Inhibitors

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Calsequestrin 2 (CSQ) is a protein in heart cells that concentrates within the sarcoplasmic reticulum (SR), where it incorporates into  $\text{Ca}^{2+}$ -release complexes. CSQ has a single N-linked glycan that can be used to determine the extent of its trafficking through the secretory system, and multiple C-terminal protein kinase CK2 sensitive phosphorylation sites. Recent data suggests that polymerization of CSQ, as well as its phosphorylation, may lead to the protein's retention within secretory compartments. In this study CSQ was conjugated to the tetrameric fluorochrome DsRed (CSQ-DsRed) and overexpressed in cultured cardiomyocytes for increasing amounts of time. Fluorescence was observed initially around myonuclei and increased distally with longer periods of incubation. High specificity anti-DsRed antibody did not detect perinuclear CSQ-DsRed, but instead stained junctional SR puncta. These findings suggested that tetrameric CSQ-DsRed was retained around nuclei following its translation, after which it trafficked anterogradely in monomeric form. Additionally, we demonstrated that CSQ phosphorylation could be modified either genetically or pharmacologically to alter its retention in the secretory system. Point mutations (<sup>378, 382, 386</sup>Glu and <sup>378, 382, 386</sup>Ala) were made to canine CSQ phosphorylation sites, mimicking either constitutive phosphorylation (CSQ-aPP) or dephosphorylation (CSQ-nPP), respectively. Mannose trimming of CSQ's glycan was increased (showing increased trafficking) or decreased (showing decreased trafficking) for overexpressed CSQ-nPP or CSQ-aPP, respectively. The CK2 specific inhibitor tetrabromocinnamic acid (TBCA) also inhibited phosphorylation in cultured cells, and produced glycans with increased mannose trimming compared to control. Supported by previous data showing a decrease in CSQ glycan mannose trimming in hypertrophic tissue, we hypothesize that a phosphorylation-dependent perinuclear shift in CSQ localization may accompany cardiac hypertrophy. If this model is correct, then pharmacological inhibition of CK2 may present a novel means of prevention or reversal of hypertrophic cardiomyopathy.

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