RCAN1–Calcineurin Axis and the Set-Point for Myocardial Damage During Ischemia-Reperfusion

J. Jose Corbalan, Richard N. Kitsis

RCAN1 (regulator of calcineurin 1) is an endogenous inhibitor of the Ca^{2+}-activated protein phosphatase calcineurin. It has been known for some time that deletion of RCAN1 in the mouse exacerbates ischemia/reperfusion-induced infarction in heart[1] and brain.[2] In this issue of *Circulation Research*, Parra et al[3] elucidate an underlying mechanism.

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Calcineurin is a widely expressed serine/threonine phosphatase consisting of 1 catalytic and 1 regulatory subunit.[4] This enzyme is activated by binding of Ca^{2+} to EF-hand motifs in the regulatory subunit and by calmodulin binding to the catalytic subunit. Calcineurin exerts pleiotropic effects including T-lymphocyte activation, neurite outgrowth, heart valve formation, skeletal myocyte differentiation, and cardiac and skeletal muscle hypertrophy. These effects are attributable primarily to calcineurin-mediated dephosphorylation of transcription factors (eg, NFAT [nuclear factor of activated T cells][5,6]) resulting in their nuclear translocation and activation of various gene expression programs. Calcineurin, however, also acts on other substrates including structural proteins, receptors, channels, and signaling molecules.[7]

RCAN1, encoded by a gene in the Down syndrome critical region 1 on human chromosome 21, is enriched in striated muscle and brain.[4] RCAN1 inhibits calcineurin through direct binding to its catalytic subunit. As might be expected from the actions of calcineurin, cardiomyocyte-specific overexpression of RCAN1 in mice inhibits cardiac hypertrophy elicited by pathological or physiological stimuli.[8] Less expected are effects of RCAN1 on ischemia/reperfusion injury. Cardiomyocyte-specific transgenic overexpression ameliorates myocardial damage in vivo, whereas the opposite is observed in mice with generalized RCAN1 knockout.[1] Moreover, pharmacological inhibition of calcineurin reverses this RCAN1-deficient phenotype showing that these effects of RCAN1 depletion are mediated by unleashing of calcineurin.

So, what mechanisms connect RCAN1–calcineurin signaling with myocardial ischemia/reperfusion injury (Figure)? Parra et al postulated that mitochondria may be involved. One molecule known to link calcineurin with mitochondria is DRP1 (dynamin-related protein 1)—a GTPase involved in mitochondrial fission. Calcineurin-mediated dephosphorylation of serine 637 in human DRP1 triggers DRP1 translocation from cytosol to mitochondria, where it promotes outer mitochondrial membrane constriction events involved in mitochondrial fission.[9] In fact, the investigators observed that RCAN1 depletion induces mitochondrial fragmentation in a variety of cell types, including mouse embryonic fibroblasts and primary neonatal and adult rodent cardiomyocytes—an effect that was reversed by pharmacological inhibition of calcineurin or DRP1. Moreover, DRP1-mediated mitochondrial fission may not be the only mechanism responsible for mitochondrial fragmentation because RCAN1 knockdown also results in selective decreases in OPA1 (optic atrophy 1) and, under some experimental conditions, MFN2 (mitofusin 2)—proteins that mediate mitochondrial fusion. Although the mechanisms linking RCAN1 with OPA1 and MFN2 are not known, these observations suggest that RCAN1 maintains mitochondrial connectivity through coordinate inhibition of fission and promotion of fusion.

The relationship between mitochondrial dynamics (fission and fusion) and cell death is poorly understood. BAX (BCL-2 [B-cell leukemia/lymphoma 2]–associated X) is a BCL-2 protein that mediates permeabilization of the outer mitochondrial membrane and cytochrome c release during apoptosis. Although complex interactions have been reported between DRP1 and BAX,[10] the role of mitochondrial fission in apoptosis remains unclear.[11] An additional wrinkle is that other studies suggest that promotion of mitochondrial connectivity (whether by increasing fusion or decreasing fission) sensitizes cells to Ca^{2+}-induced opening of the permeability transition pore (mPTP) on the inner mitochondrial membrane and necrotic cell death[12]—arguably of more relevance in myocardial ischemia–reperfusion injury than apoptosis.

To gain insights beyond mitochondrial dynamics, Parra et al chose to examine the role of Ca^{2+} itself in the death process and made an interesting observation: RCAN1-deficient cardiomyocytes exhibit a deficit in mitochondrial Ca^{2+} uptake and concomitant elevation of cytosolic Ca^{2+} concentrations. The mechanism by which RCAN1 depletion dampens mitochondrial Ca^{2+} uptake is not clear but may reflect observed decreases in polarization across the inner mitochondrial

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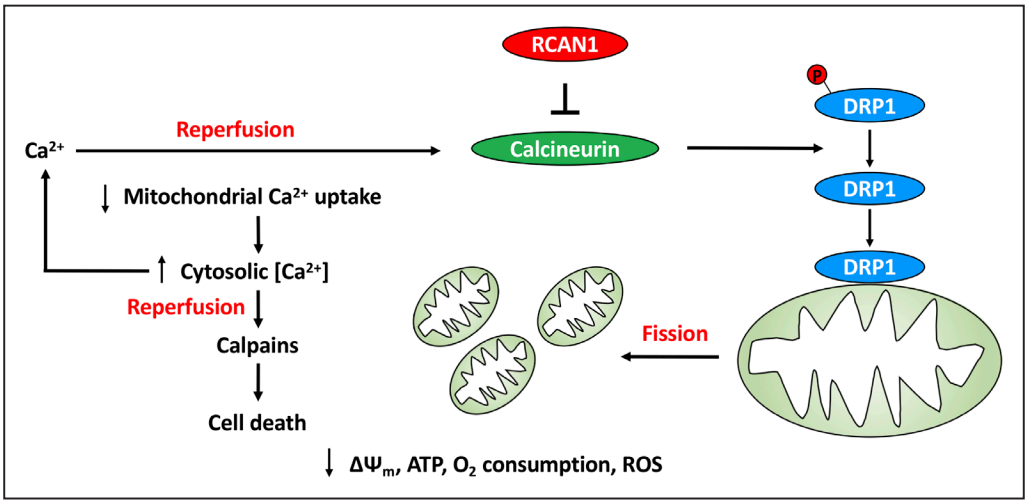

Figure. RCAN1 (regulator of calcineurin 1) modulates tissue damage during myocardial ischemia–reperfusion. Depletion of RCAN1 disinhibits the Ca^{2+}-activated protein phosphatase calcineurin resulting in calcineurin-mediated dephosphorylation of serine 637 in human DRP1 (dynamin-related protein 1). This stimulates the translocation of DRP1 from cytosol to the outer mitochondrial membrane where it promotes mitochondrial fission. Depletion of RCAN1 may also promote mitochondrial fragmentation through decreases in mitochondrial fusion proteins OPA1 (optic atrophy 1) and MFN2 (mitofusin 2) (not shown). Fragmented mitochondria manifest decreased Ca^{2+} uptake, which may reflect their loss of electric potential difference across the inner mitochondrial membrane ($\Delta\psi_m$) or perhaps a more direct action of calcineurin on the mitochondrial Ca^{2+} import machinery. Impaired uptake of Ca^{2+} results in elevation of cytosolic Ca^{2+} concentrations, thereby predisposing to the activation of calpains—Ca^{2+}-activated proteases. Calpains cleave signaling and structural proteins to induce cell death. Activation of calcineurin and calpains in this schema takes place during reperfusion because the activities of these enzymes are inhibited in the acidic environment of ischemia.

membrane ($\Delta\psi_m$)—a driving force in the movement of Ca^{2+} into the mitochondrial matrix through the mitochondrial calcium uniporter.[13] This begs the question, however, as to why $\Delta\psi_m$ is decreased in RCAN1-depleted cardiomyocytes. One possibility is that decreases in $\Delta\psi_m$ reflect merely the poor health of the fragmented mitochondria, which also exhibit decreased ATP levels. Another possibility is that RCAN1 deficiency somehow causes a primary defect in mitochondrial Ca^{2+} uptake which, in turn, results in decreased catabolism of nutrients. In fact, lower rates of oxygen consumption and ROS (reactive oxygen species) production were observed in RCAN1-depleted cells. Further study will be needed to test whether the RCAN1–calcineurin axis directly regulates mitochondrial Ca^{2+} uptake.

Although decreased mitochondrial Ca^{2+} uptake could compromise substrate oxidation in mitochondria, it would be expected to be protective against Ca^{2+}-induced opening of the mPTP and necrosis. What then would account for the increased sensitivity to cell death in RCAN1-deficient cardiomyocytes? Given the relative increases in cytosolic Ca^{2+}, the investigators considered Ca^{2+}-dependent mechanisms that may be operating in this cellular compartment. In fact, they observed activation of calpains—Ca^{2+}-stimulated proteases whose substrates include signaling and structural molecules involved in apoptotic and necrotic cell death.[14,15] Importantly, combined knockdown of calpains 1 or 2 or pharmacological inhibition of these proteases reversed the increased sensitization to simulated ischemia–reperfusion-induced cell death resulting from RCAN1 knockdown.

Thus, in essence, what RCAN1 deficiency seems to be doing is to shift the dominant cell death mechanism during ischemia–reperfusion away from the mPTP and toward cytosolic calpain activation. The augmentation of cell killing resulting from this shift may reflect the fact that high Ca^{2+} concentrations are less well tolerated in the cytosol than in the mitochondrial matrix.

The investigators also explored the effects of increasing RCAN1 levels. High levels of RCAN1 overexpression from adenoviral-mediated transduction of cardiomyocytes resulted in hyperconnected mitochondria with augmented rates of oxygen consumption and ROS levels. Moreover, in keeping with the cardioprotection afforded by transgenic overexpression in cardiomyocytes in vivo, RCAN1 overexpression ameliorated cardiomyocyte death elicited by simulated ischemia–reperfusion. Similar findings were obtained with more mild overexpression of RCAN1 as observed in cardiomyocytes derived from trisomic 21 human-induced pluripotent stem cells compared with cardiomyocytes derived the same induced pluripotent stem cells in the disomic state (the extra chromosome 21 being spontaneously lost with passage in culture). Importantly, the phenotype in the trisomic cells is attributable specifically to RCAN1—rather than the extra copy of multiple other genes on chromosome 21—because it is reversed by RCAN1 knockdown. Moreover, these findings suggest that excess RCAN1 is an important factor in the high degrees of oxidative stress present in the cells of patients with Down syndrome.

In summary, this study establishes the importance of RCAN1 in maintaining a connected mitochondrial network. In addition, it delineates a critical mechanism by which the RCAN1–calcineurin axis regulates the susceptibility of the myocardium to damage from ischemia–reperfusion (Figure). Future work is needed to elucidate the precise molecular connections by which calcineurin impacts mitochondrial Ca^{2+} uptake. In addition, the existence of this mechanism does not preclude the possibility that other calcineurin substrates contribute to the set point of

tissue damage resulting from ischemia–reperfusion. Finally, the possibility that this mechanism also operates during ischemia–reperfusion in the brain merits further investigation.

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Disclosures

None.

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