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MEETING ABSTRACT

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Adenosine kinase mediates adenosine attenuation of cardiomyocyte microtubule cytoskeletal densification

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Background: Microtubules play essential roles in cell size, shape and intracellular trafficking. In the heart however, extensive densification of the cardiomyocyte microtubule cytoskeleton under hypertrophic stress conditions is associated with contractile dysfunction. Myocardial adenosine attenuates cardiomyocyte microtubule densification, hypertrophy and heart failure in the setting of pressure overload, but the mechanism(s) by which adenosine regulates microtubule dynamics is not clear. Here we investigated the role of adenosine receptors and intracellular metabolism by adenosine kinase (ADK) in adenosine regulation of cardiomyocyte microtubule dynamics.

Methods: Cultured neonatal rat ventricular cardiomyocytes (NRVMs) were stimulated with phenylephrine (50 µM) or constitutively activated Raf+Akt (10 MOI/cell) to induce hypertrophic growth and microtubule densification. To examine the impact of adenosine receptors or adenosine metabolism in adenosinergic effects, NRVMs were further treated with adenosine (10 µM; plus adenosine deaminase inhibitor pentostatin (1 µM)) or 2-chloroadenosine (CADO; 5 µM) in the presence of selective adenosine receptor antagonists (1–5 µM) or ADK inhibitors (iodotubercidin (0.2 µM) or ABT-702 (0.2 µM)). In addition, ADK or 5′-cytoplasmic nucleotidase (5′cNi) were over-expressed to examine the impact of adenosine conversion to AMP. Microtubule dynamics, cell signaling, and cell morphology were analyzed by subcellular fractionation, western blot and immunofluorescence.

Results: Phenylephrine or Raf/Akt caused cardiomyocyte microtubule stabilization and hypertrophy. Both of these processes were attenuated by adenosine or CADO. While adenosine receptor antagonists only modestly blocked adenosine effects on microtubules, adenosine kinase inhibitors or expression of 5′cNi potently reversed microtubule destabilization by adenosine and restored cardiomyocyte hypertrophy. Conversely, ADK over-expression potentiated adenosine destabilization of microtubules. Remarkably, adenosine attenuated microtubule stabilization and cardiomyocyte hypertrophy in Raf/Akt-transfected cells despite unmitigated mTORC1 and ERK pathway signaling. The ADK dependent destabilization of microtubules by adenosine was not associated with increased activation of AMPK.

Discussion: Intracellular conversion of adenosine to AMP attenuates microtubule stabilization and cardiomyocyte hypertrophy independent of AMPK, even in the setting of constitutive hypertrophic signaling. ADK attenuation of microtubule cytoskeletal network expansion may serve to limit cardiomyocyte growth during metabolic stress conditions.

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