Peroxynitrite Biochemical Signaling Regulates miR21 Activation of NLRP3 Inflammasome and Is Key to PCOS Severity

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Biochemical signaling plays a key role in molecular pathways in various disease processes of women's reproductive health. Polycystic Ovarian Syndrome (PCOS) affects 66 million reproductive-age women worldwide with no definite treatment regimen in place. I hypothesize that peroxynitrite, a strong cellular oxidant will trigger the activation of micro-RNA (miR)-21 that ultimately will activate inflammasome in mouse organoids and human granulosa (KGN) cells. Using both a novel mouse ovarian organoid system and an in vitro cellular model of human origin, I show that both systems produced a significant increase in miR21 and a parallel increase in ONOO-. To prove the role of biochemical redox pathways, I used DMPO (nitrone spin trap), Apocynin (NOX2 enzyme inhibitor) and Phenyl Boronic Acid (PBA)(peroxynitrite scavenger). Results showed that specific inhibitors of peroxynitrite pathway significantly decreased miR21 levels, suggesting that ONOO- is involved in miR21 activation. It also showed that ONOO- resulted from an activated NOX2 enzyme since apocynin, an inhibitor of p47 phox translocation to the membrane blocked ONOO- formation. To study whether ONOO- also modulated the downstream inflammasome activation, I conducted dual labelled immunofluorescence microscopy for visualizing NLRP3-ASC2 colocalization, Further, ONOO- inhibitors significantly decreased inflammasome activation and release of IL1 beta, a secreted proinflammatory cytokine. In conclusion, my data represented a unique and novel redox mediator in PCOS that was upstream of the miR21-NLRP3 axis and may be used as a therapeutic target, with PBA being a novel treatment for PCOS.

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