ABSTRACTS

Exploring cell-free mitochondria as a potential non-invasive biomarker of lupus nephritis

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Background: Systemic lupus erythematosus (SLE) is a systemic chronic autoimmune disease. Lupus nephritis (LN) is one of the major manifestations of SLE affecting up to ~60% SLE patients. According to European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR), a positive renal biopsy along with a positive antinuclear antibody (ANA) and/or anti-double-stranded DNA (anti-dsDNA) antibody is required for confirmation and classification of LN. Renal biopsy is the gold standard to diagnose and classify LN, but it is invasive and costly. Therefore, there is a need for less invasive, risk-free systemic biomarkers to predict disease activity and treatment outcomes. Cells under oxidative stress can release various Mitochondrial Danger Associated Molecular Patterns (DAMPs) including naked or vesicle-enclosed forms of mitochondria itself known as cell-free mitochondria (cf-mitochondria), mitochondrial DNA etc. Stressful conditions are also well known to cause mitochondrial extrusion by damaged organs. The cf-mitochondria can act as auto-antigen, thus triggering immune response leading to production of anti-mitochondrial DNA autoantibodies.

Methods: The study recruited 39 SLE patients and 37 gender and age-matched, healthy controls. Healthy controls had normal kidney function with no known chronic disease or ongoing infection. Blood and urine samples were collected from the recruited individuals after obtaining informed written consent. This study has evaluated various DAMP entities released by mitochondria, including cell-free mitochondria, cell-free mitochondrial DNA, and autoantibodies against mitochondrial DNA, as probable biomarkers of the degree of nephritis. Two forms of the cell-free mitochondria including vesicle-enclosed and naked ones were enumerated in plasma by FACS using vesicle-specific FM-4-64 and mitochondria-specific Mitotracker Green FM dyes. Total cell-free urinary DNA (u-cfDNA) was isolated using commercially available MagMAXTM Cell-Free DNA Isolation Kit (Applied Biosystems, USA). Isolated u-cfDNA was quantified for both nuclear and mitochondrial-origin DNA using the SYBR-based qPCR. anti-Mitochondrial DNA specific autoantibodies by ELISA using the purified mtDNA as coating antigen.

Results: SLE patients' blood had significantly high levels of cell-free mitochondria. High levels of urinary cell-free mitochondrial DNA are present in SLE patients. High titer of autoantibodies against mitochondria is present in the plasma of SLE patients. All three mitochondria-associated markers, i.e. cell-free mitochondria, cell-free mitochondrial DNA, and autoantibodies against mitochondrial DNA showed a significant correlation with proteinuria and protein creatinine ratio.

Conclusion: The current study substantiates the usage of mitochondrial DAMPs-based readouts as clinical biomarkers of nephritis upon their validation in a larger cohort of lupus nephritis patients and other forms of nephritis.

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