The regulation of skin fibrosis in systemic sclerosis by extracellular ATP via P2Y2 purinergic receptor.

Objective: Systemic sclerosis (SSc) is a connective tissue disorder characterized by the development of fibrosis in the skin and internal organs as well as microvascular dysfunction. There is growing evidences that vasculopathy-induced hypoxia and oxidative stress enhances the process of fibrosis in SSc. Tissue injury/hypoxia and oxidative stress induced-extracellular ATP can act as a damage-associated molecular pattern molecules (DAMPs), which initiates inflammatory response. Regarding ATP and tissue fibrosis, several studies has been reported that extracellular ATP induced the development of fibrosis in several organs, such as heart, lung, liver and kidney. However, the role of ATP in the pathogenesis of skin fibrosis in SSc is unclear. Objective was to elucidate the role of extracellular ATP in skin fibrosis in Systemic sclerosis (SSc).

Methods: ATP-induced IL-6 mRNA/protein levels in normal and SSc fibroblasts were compared. The effect of several antagonists of purinergic receptors and p38 inhibitors on ATP-induced IL-6 production in SSc fibroblasts were analysed. The effect of P2Y2 purinergic receptor antagonist on Bleomycin-induced dermal fibrosis in mice was investigated.

Results: We identified that hypoxia induced ATP release from SSc fibroblasts and normal
human endothelial cells. In addition, we determined that ATP enhanced IL-6 production in normal and SSc fibroblasts, and that ATP-induced IL-6 production was significantly higher in SSc fibroblasts than in normal fibroblasts. The expressions of purinergic P2X and P2Y receptors were not changed between normal and SSc fibroblasts, suggesting that purinergic P2 receptors expression might not be associated with the mechanisms underlying the higher ATP-induced IL-6 production observed in SSc fibroblasts.

Additionally, non-selective P2 receptor antagonist, Suramin, and P2Y$_2$ receptor antagonist, Kaempferol and AR-C118925XX, significantly suppressed the ATP-induced IL-6 production in SSc fibroblasts. ATP-induced IL-6 production was also significantly inhibited by p38 inhibitors, SB203580 and Doramapimod. These results suggest that ATP-induced phosphorylation of p38 via purinergic P2Y$_2$ receptor might enhance IL-6 production in the SSc fibroblasts. Collagen type I production in SSc fibroblasts by ATP-induced IL-6/IL-6 receptor trans-signaling was inhibited by Kaempferol and SB203580. These results suggest that ATP-induced IL-6 from dermal fibroblasts may have both paracrine and autocrine effects on dermal fibroblasts, T cells and B cells, and result in the skin fibrosis in SSc. Combined treatment with ATP and norepinephrine or ET-1 resulted in an additive increase in the production of IL-6 in the SSc fibroblasts.

ATP is converted to AMP and adenosine by ectonucleotidase CD39 and CD73. We found that CD39 was not expressed in normal and SSc fibroblasts. In addition, CD73 and adenosine receptor expressions were not changed between normal and SSc fibroblasts. These results suggest that ATP-adenosine conversion may not be associated with ATP-induced IL-6 production in SSc fibroblasts \textit{in vitro}.

We found that the amount of ATP in the mouse skin was significantly enhanced by injection of bleomycin for 7 days, and administration of Kaempferol, significantly inhibited Bleomycin-induced dermal fibrosis in mice.
Conclusions: These results suggest that vasculopathy-induced hypoxia and oxidative stress might enhance ATP release in the dermis in SSc, and extracellular ATP-induced phosphorylation of p38 via P2Y_2 receptor might enhance IL-6 and collagen type I production in SSc fibroblasts. P2Y_2 receptor antagonists therapy could be an alternative treatment for skin sclerosis in patients with SSc.