Certificate

This is to certify that the research work **"Understanding the regulatory role of PAD-4 on Del-1 and evaluation of PAD-4 inhibitors in pulmonary fibrosis"** is conducted by Biswajit Panda. It is a bonafide and original research work carried out by him under my guidance at NIPER Hyderabad.

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30/08/2024

Citation (summary) of the research work

Exaggerated NETosis triggered in neutrophils by the prolonged action of the enzyme peptidyl arginine deiminase-4 (PAD-4) due to recurrent micro-injury to the lungs initiate inflammatory and fibrotic cascade of reaction progressing to pulmonary fibrosis (PF). Standard PAD-4 inhibitor chloro-amidine (CLA) led to significant decrease in inflammatory, fibrotic and NETotic cytokines and markers. PAD-4 inhibition also resulted in increase in levels of an anti-inflammatory mediator Del-1, paving way for a novel therapeutic option and combination treatment. In addition to neutrophils, macrophages also play a strategic role in progression of inflammatory and fibrotic events in PF. Macrophages are cells possessing a high level of plasticity where they polarise into M1 phenotype during the initial inflammatory phase and M2 phenotype during the late fibrotic phase of PF progression. The M1 macrophages are pro-inflammatory and secrete inflammatory mediators while the M2 macrophages are anti-inflammatory and pro-fibrotic in nature that secrete growth factors and fibrotic mediators. We have also demonstrated that CLA resulted in time dependent amelioration of PF with reduction of M1 macrophage markers and released cytokines during initial inflammatory phase and M2 macrophage and released fibrotic mediators during the late fibrotic phase. The significance of PAD-4 as a molecular target in PF progression has propelled us to synthesise novel PAD-4 inhibitors and screen them for their anti-fibrotic effects in *in vitro* and *in vivo* models of PF. We have synthesised a total of 23 indole-pyrazolopyrimidine derivative capable of inhibiting PAD-4. Upon evaluation of anti-NETotic effects, we observed that the NCEs were significantly inhibiting major NETotic markers including PAD-4 at mRNA and protein levels. We isolated the NETs from the neutrophils and exposed them to fibroblasts to evaluate the anti-fibrotic effects of treated NETs. The *in vitro* anti-fibrotic studies demonstrated effective inhibition of the major fibrotic markers at mRNA and protein levels. The significant inhibition of NETs and fibrosis observed in cell based study led us to carry out the *in vivo* anti-fibrotic evaluation in bleomycin induced PF mice model. The selected PAD-4 inhibitor showed normalisation of the lung functional parameters. It ameliorated the abnormal BALF measurements and higher total neutrophils produced in the body. Additionally, it also reduced the expression of the major pro-inflammatory cytokines, oxidative stress markers, EMT markers, ECM proteins, markers for matrix remodelling and fibrogenesis and NETotic markers. These studies affirmed the potential of PAD-4 as a target for inflammatory disorders.

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