Development of a high-throughput screening assay to discover protein-protein interaction disruptor compounds as a novel anti-fibrosis treatment.

Introduction: Mechanisms that regulate fibrinolysis after injury are important for tissue repair. A number of pathological conditions, such as atherosclerosis, multiple sclerosis and pulmonary disease, are characterised by defective fibrin degradation. A TNF receptor superfamily member, p75 neurotrophin receptor (p75NTR), enhances the degradation of cAMP in its vicinity via protein-protein interaction (PPI) with phosphodiesterase PDE4A4. This PPI is responsible for a decreased extracellular proteolytic activity; a limiting factor for tissue repair. So far a number of PDE4-specific inhibitors have been generated, but they lack specificity and exhibit mechanism-associated side effects.

Methods: To increase regenerative capacity and avoid unwanted side effects, we focused on the disruption of the interaction between p75NTR and PDE4A4. We developed an ELISA high-throughput screening assay as part of the drug discovery process for PPI disruptor compounds that would unhook the PDE4A4-p75 complex. We optimised the production of PDE4A4 protein and evaluated the conditions of the interaction suitable for an appropriate assay. We then screened 4,040 compounds and identified 45 “hits” from a proprietary compound library.

Results: Some of the compounds are structurally related analogues to moexipril, a well tolerated and approved angiotensin converting enzyme (ACE) inhibitor. Moexipril was recently identified, using a computational approach, as a potential PDE4 inhibitor. Finally, 20 of the 'hits' were selected for screening validation based on a fibrinolysis assay.

Conclusions: After further validation, we expect to identify a novel compound for a targeted and well tolerated treatment for a number of diseases characterised by defective tissue repair.