The role of extracellular matrix protein 1 (ECM1) - a novel link between inflammation and cardiac fibrosis

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Introduction: Cardiac fibrosis is a severe consequence of cardiovascular disease and aging, in which we currently have no effective treatments. The mechanisms underpinning the development of cardiac fibrosis remains poorly understood. Our preliminary data suggested extracellular matrix protein 1 (ECM1) is involved in cardiac fibrosis. We therefore aimed to investigate the role of ECM1 in several fibrotic cardiac diseases.

Methods: Young and ageing (3m/18m) male C57BL/6 mice, and primary mouse cardiac fibroblast (cFB) cultures, commercial human cardiac fibroblasts (Hu-cFB), human coronary artery endothelial cell (HCAEC)/smooth muscle cell (HCASMC), and human cardiac myocyte (HCM) cell lines were used. Young mice were subject to myocardial infarction (MI, 3-day/28-day, n=6/6), or pressure overload (TAC, 3-day/13-week, n=4/4). Left ventricle (LV) was collected at all time-points, and at 18m (ageing; n=3). Spleen and bone marrow was extracted from young control mice. Hu-cFB cells were treated with recombinant ECM1 (20ng/ml) for either 10, 30 or 50 min, or 48h. Immunoblotting was conducted on all samples, qPCR on LV tissue only, density gradient centrifugation and multicolour flow cytometry coupled with fluorescent ECM1 in-situ hybridisation (FISH-Flow) on bone marrow cells.

Results: ECM1 expression was upregulated in ageing LV (mRNA 2.2±0.1-fold, p=0.0002; protein 2.0-fold, p=0.0006), day-3 post-MI (mRNA, 4.9±2.0-fold, p=0.004; protein, 3.0-fold, p=0.004), a trend of ECM1 upregulation was observed at day-28 post-MI (mRNA, 13.2±12.0-fold, p=0.003; protein, 1.8-fold, p=0.2), but no change post-TAC. Both ERK1/2 and AKT phosphorylation was upregulated 10 min post-ECM1 treatment of Hu-cFBs (ERK1/2, 2.0-fold, p<0.0001; AKT, 1.9-fold, p<0.0001), and Collagen-I protein expression was upregulated 48h post-ECM1 treatment (1.9-fold, p=0.004). ECM1 protein was not expressed in cFB, Hu-cFB, HCAEC, HCASMC or HCM, however ECM1 protein was highly expressed in spleen and bone marrow; to a greater extent in granulocytes compared to monocytes (p=0.004). tSNE analysis of ECM1 mRNA FISH-Flow revealed ECM1+ are highly granular, moderate to large in size, and express (to varying levels) CD45, CD11b, CD11c, F4/80, Ly6-C, Ly-6G, and FcerI-a. However ECM1+ cells did not express markers indicative of smaller cells (CD3 or MHC II).

Conclusions: These data demonstrate that ECM1 plays a role in ageing and post-MI fibrosis. Although ECM1 was not produced by resident cardiac cells, it was highly expressed in spleen and bone marrow; specifically,
large, granular bone marrow cell sub-types such as granulocytes and/or macrophages. Our data suggest ECM1 is expressed by cardiac infiltrating leukocytes to provoke fibroblast collagen expression in a disease specific manner; potentially via the ERK1/2 and/or AKT pathway activation. Therefore, ECM1 warrants further investigation, and may be a promising target for the treatment of fibrotic cardiac diseases.