[gw22-e0405] **TISSUE KALLIKREIN INCREASE ENDOTHELIAL PROGENITOR CELLS VIA B2-PI 3-KINASE/AKT PATHWAY**

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**Objective** Tissue kallikrein (TK) has been demonstrated to improve neovasculogenesis after myocardial infarction (MI). In the present study, we examined whether TK plays a role in the peripheral endothelial progenitor cells (EPCs) function and its underlying mechanisms in the TK-induced elevation of EPCs.

**Methods** Peripheral blood derived mononuclear cells containing EPCs were isolated from rat, and then the cells were cultured on fibronectin-coated tissue culture plates. After seven days of culture, the differentiated EPCs were characterised as cells double-positive for DiI-LDL uptake and UEA-lectin binding under a fluorescent microscope. The in vitro effects of TK on EPC differentiation, apoptosis, migration, and vascular tube formation capacities were studied, in the presence or absence of TK, B2 receptor antagonist Icatibant, phosphatidylinositol-3-kinase inhibitor LY294002. Apoptosis was evaluated by FACS analysis using Annexin V-FITC/PI staining. Akt phosphorylation and cleaved caspase-3 were measured by Western blot analysis. The effects of human tissue kallikrein gene adenoviral vector (Ad.hTK) administration on the number of $CD34^+FLK\text{-}1^+$ progenitors in the peripheral circulation, vasculogenesis and cardiac function were then assayed in the MI mouse model.

**Results** Administration of TK significantly increased the number of DiI-LDL/ UEA-lectin double positive early EPCs as well as in their migration, tube formation properties in vitro. Transduction of TK in cultured EPCs attenuated apoptosis induced by hypoxia. TK treatment led to an increase in the phosphorylation of Akt and a decrease of cleaved caspased-3. The beneficial effects of TK could be blocked by pretreatment with Icatibant, LY294002. The expression of recombinant human TK in mouse heart significantly improved cardiac contractility (shown by ejection fraction 38%±6.9% vs 24.6%±4.9%, $p<0.01$, n=5), reduced infarct size seven days after gene delivery (17.9%±6.1% vs 39.5%±9.1%, $p<0.01$). Compared with the Ad. Null group, Ad.hTK significantly increased the number of circulating $CD34^+FLK\text{-}1^+$ EPCs (4.89%±3.8% vs 15.1%±1%, $p<0.01$) and promoted the growth of capillaries (100±16 vs 197±10 capillaries/$mm^2$, $p<0.05$) and arterioles (50.8±7 vs 111.6±24 arterioles/$mm^2$, $p<0.05$) in the peri-infarct myocardium.

**Conclusion** These data provide the direct evidence that TK promotes vessel growth by increasing the number of circulating EPCs and enhancing their functional properties through the bradykinin B2 receptor-Akt signalling pathway.