

On the mechanism of angiogenesis induced by thrombin receptor activating peptides (TRAP-9)

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Angiogenesis and vascularization is a critical issue in tissue engineering. Thrombin, a serine protease, plays an important role in inflammation and injury. It is present in the site of inflammation and activates platelet and different cellular response including angiogenesis. It interacts with Protease-activated receptors (PAR) on the endothelial cell surface and cleaves a terminal portion of the receptor which new N-terminal autostimulates the receptor and cause transmembrane signaling. Synthetic peptide containing agonist motif such as SFLLRNCCC called thrombin receptor activating peptide (TRAP-9)

for human PAR-1, capable of causing full receptor activation and mimic many effects of thrombin. In the current study, we investigate the maximum concentration of TRAP-9 which is capable of Human umbilical vein endothelial cell (HUVEC) proliferation. Different HUVEC cell marker's expression after stimulation with TRAP-9 and thrombin were determined. Also, the effect of TRAP-9 on vascular network formation in HUVEC/ASC co-culture was assessed.

Method: In this study, thrombin receptor activating peptide (TRAP-9) with motif SFLLRNCCC (Ser, Phe, Leu, Leu, Arg, Asn, 3*Cys) was synthesized. powder was diluted with PBS and frozen at -20°C. Confluent HUVEC was treated with different TRAP-9 concentration and proliferation after 24hrs we checked with ELISA. Different HUVEC cell marker was determined using flow cytometry after 24 stimulation with TRAP-9 and thrombin. Finally, the effect

of TRAP-9 on vascular tube formation in GFP-HUVEC/ASC co-culture was assessed and networks were quantified using AngioSys.

Result: Proliferation of HUVEC enhanced with TRAP-9 which 500µM showed higher proliferation compare to lower concentrations. HUVECs markers expression level is in a similar amount as thrombin. A number of junctions, tubules and total tubular length improved when cells treated with TRAP-9.

Conclusion: Data showed that TRAP-9 is capable of HUVEC proliferation without toxic effect even in high concentration. Level of cell markers expression is comparable with thrombin. Incorporation of TRAP-9 in fibrin clot co-culture indicates that this motif improved networks formation and would be a promising agonist for thrombin for angiogenesis.

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