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

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Abstract

Angiogenesis and vascularization is critical issue in tissue engineering. Thrombin, a serin protease, plays an important role in inflamation and injury. It is present in site of inflammation and activates platelet and different cellular response including angiogenesis. It interact with protease activated receptors (PAR) on endothelial cell surface and cleaves terminal portion of receptor which new N-terminal autostimulates the receptor and cause transmembrane signaling. Synthetic peptide containing agonist motif such as SFLLRNCCC called thrombin receptor activating preptides (TRAP-9) for human PAR-1, capable of causing full receptor activation and mimic many effects of thrombin. In current study, we investigate the maximum concentartion of TRAP-9 which is capbale of human umbilical vain endothelial cell (HUVEC) proliferation. Diffreent HUVEC cell marker´s expression after stimulation with TRAP-9 and thrombin were determined. Also 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 synthetized.poweder was diluted with PBS and and freezed at -20^{oc}. confluent HUVEC was treated with different TRAP-9 concentartion and proliferation after 24h wa checked with ELISA.Different HUVEC cell marker was determined using flow cytometry after 24 stimulation with TRAP-9 and thrombin.Finally 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 concentartions.HUVECs markers expression level is in similar amount as thrombin. Number of junctions,tubuls and total tubul length improved when cells terated with TRAP-9.

Conclusion

Data showed that TRAP-9 is capable HUVEC proliferation without toxic effect even in high concentartion.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 promissing agonist for thrombin for angiogenesis.

Key words: HUVEC/ASC co-culture,TRAP-9,PAR-1,angiogenesis,thrombin

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Biography

Mahdieh Heydarigoojani studied Biomedical engineerinmg and intrested in improvement of angiogenesis and vascularization in tissue engineering field. She graduated in 2014 and recived her bachelor in

Biomedical engineering. She intended to improve her knowledge and accepted at technical university of Vienna, studied Biomedical engineering in master. Due to personal passion for tissue engineering, she decided to work in this field and started her master thesis in tissue engineering with focus on improvement of vascularozation at LBI institute. She would like to continue her carrier in this way.

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