Thrombin stimulates expression and secretion of Monocyte chemotactic protein-1 (MCP1) and inhibit angiogenesis in extravillous trophoblast

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Introduction: Trophoblast invasion is the key step of the human embryo for successful pregnancy establishment. During subsequent development of the placenta, extravillous cytotrophoblasts (CTBs) from anchoring villi invade the decidualized endometrium and migrate into the spiral arteries (endovascular trophoblasts) (Pijnenborg et al., 1983). Trophoblast invasion and arterial remodelling extend through the depth of the decidua in a normal pregnancy. Shallow trophoblast invasion and defect in spiral artery associated with pregnancy pathologies especially preeclampsia and fetal growth restriction which are major cause of female infertility.

Monocyte chemotactic protein-1 (MCP-1) is a key CC chemokine responsible to play an integral role in the control and maintenance of a normal pregnancy from implantation to parturition (Denison et al., 1998). Thrombin is a multifunctional serine protease that plays crucial role to activate blood platelets and elicits multiple effects on a variety of cell types including extravillous trophoblast cells, vascular smooth muscle cells, endothelial cells, monocytes, cytokine release, proinflammatory activity, chemotaxis, mitogenesis, apoptosis and angiogenesis on a variety of cell types (Coughlin, 2000; Leonard et al., 2013; O'Brien et al., 2000; Popovic et al., 2012; Tsopanoglou and Maragoudakis, 2009; Zhao et al., 2012).

The aim of this study to explored Thrombin dependent regulation of MCP1 in HTR-8/SVneo cell that possibly regulate physiological functions of placental cell via cytokine secretion

Methods:

- Cell Culture,
- Tube formation assay,
- ELISA,
- MCP-1 silencing,
- RNA isolation and real time PCR

Results:

- In HTR-8/SVneo cells, MCP-1 was secreted out after Thrombin stimulation in a concentration of 0.5U/ml.
- Thrombin exclusively regulates secretion of MCP-1 in HTR-8/SVneo trophoblast cells. Interestingly, we found that Thrombin regulates MCP-1 protein secretion in 6 hours and gene expression in 12 hours.
- Moreover, MCP-1 secretion was controlled by Rac-1 and Rho/Rho kinase in 12 hours of treatment.
- The angiogenesis activity of HTR-8/SVneo cells is regulated by Thrombin via MCP-1 dependent manner.

Conclusion:

The data reported here provide evidence that Thrombin might apparently have negative impact on the ability of Endovascular Trophoblast to generate the capillary system which seems to be regulated by MCP-1.

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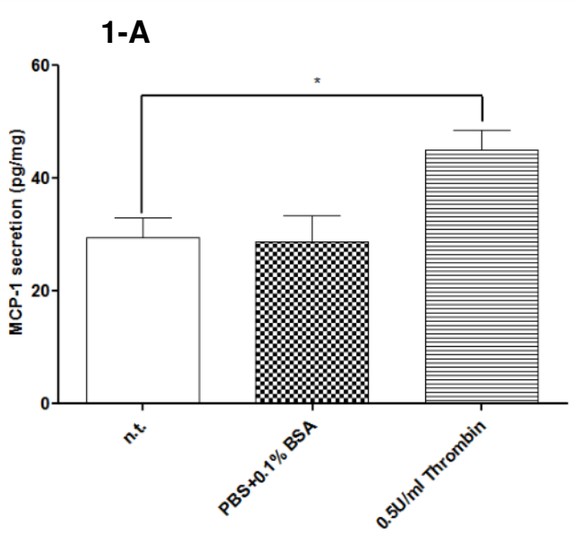


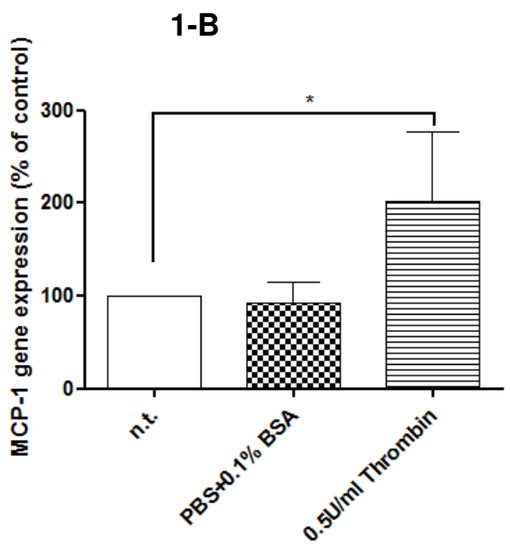


Fig.1-A .MCP-1 secretion in HTR-8/Svneo cell line. **B**-MCP-1 gene expression analysis after Thrombin stimulation in a concentration of 0.5U/ml.

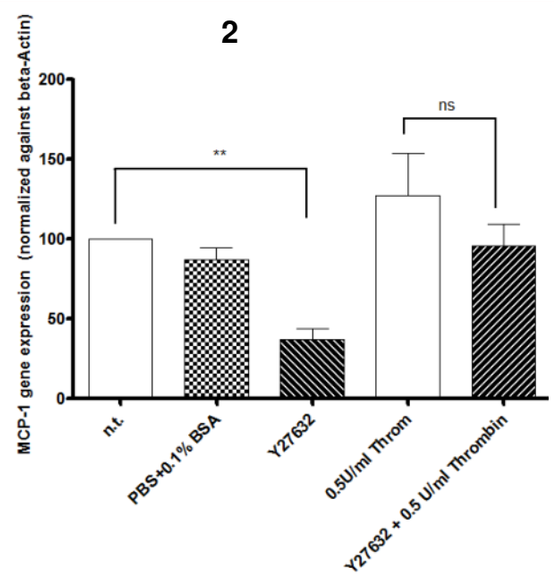
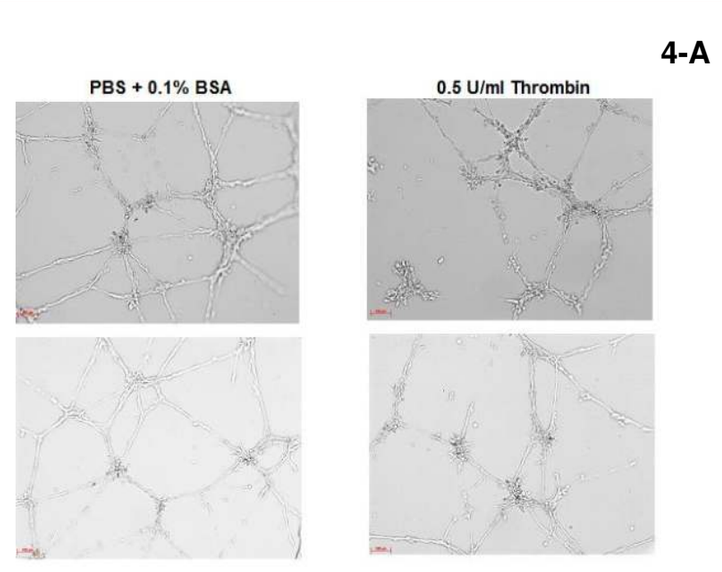
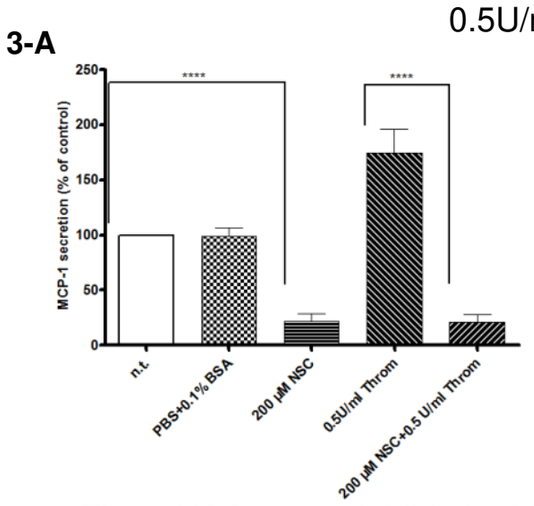


Fig. 2.Y27632 inhibit the MCP-1 gene expression in HTR-8/Svneo cell line.





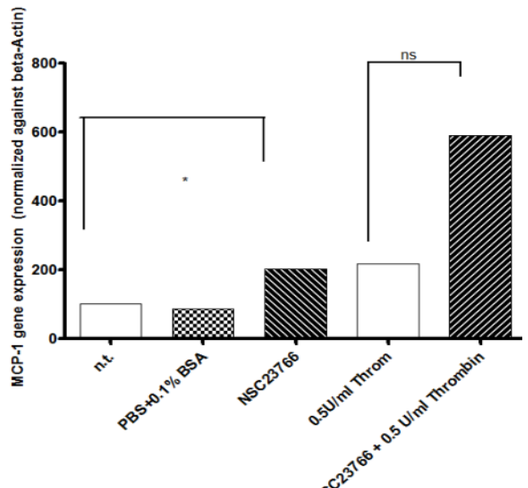


Fig. 3.NSC-23766 inhibit the MCP-1 secretion(3A) and gene expression(3B) in HTR-8/Svneo cell line.

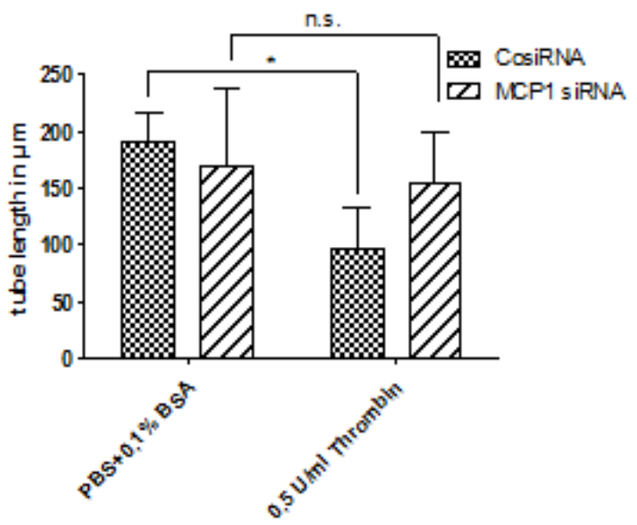


Fig. 4 (A and B) Thrombin stimulation decreased the tube length in HTR-8/Svneo cell line.

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