Cancer cell-derived microparticles expressing tissue factor have pivotal role on the procoagulant shift of endothelial cells

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Introduction

Endothelium activation is essential in pathogenesis of cancer associated thrombosis (CAT). Endothelial cell (EC) is a potential target of cancer cell derived microparticles (CaCe-dMPs). We recently showed that endothelial cells exposed to CaCe-dMPs acquire a procoagulant phenotype characterized by an enhancement of thrombin generation (TG). This new property is transferable to daughter cells.

Aim

To elucidate some aspects of the interplay between endothelial cells and cancer, we investigated whether CaCe-dMP could alter the hemostatic balance of endothelial cells and induce acquisition of procoagulant properties and if this acquisition was transferable to daughter cells. In this study, we investigated the implication of tissue factor (TF) in the new procoagulant profile acquired by EC exposed to CaCe-dMPs and if TF alone is capable of inducing this change.

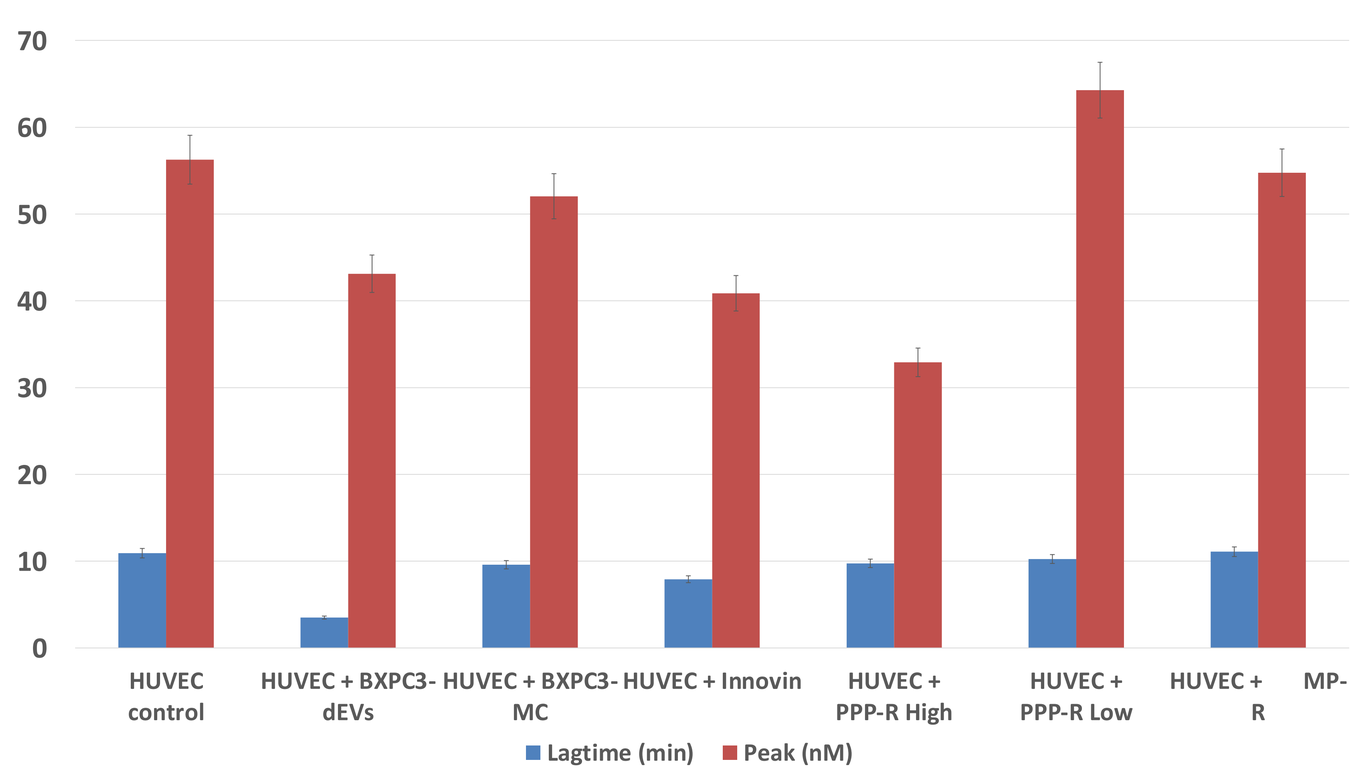
Materials & Methods

CaCe-dMP released in conditioned medium from pancreas adenocarcinoma cells (BXPC3) were isolated with differential centrifugation. Human umbilical vein endothelial cells (HUVEC) were cultured for 72h according to 5 experimental conditions: in presence of (a) BXPC3-dMPs (b) BXPC3 cell conditioned medium depleted in BXPC3-dMPs (c) no TF and 4 µM of phospholipids MP-R (d) 5pM of TF and 4 µM of phospholipids PPP-R High (e) 1pM of TF and 4 µM of phospholipids PPP-R Low or (f) 5nM recombinant TF (rTF Innovin). Subsequently, exposed-EC were washed and re-suspended in platelet poor plasma (PPP). Capacity of exposed-EC to enhance thrombin generation in PPP was assessed with the Calibrated Automated Thrombogram assay (Thrombinoscope b.v, Diagnostica Stago, Asnières, France). TF concentration of exposed cells was determined by using the Zymutest total TF kit (Hyphen Biomed, France).

Results

HUVEC exposed to BXPC3-dMPs acquired a procoagulant profile with a significant enhancement of thrombin generation as compared to control experiment (non-exposed HUVEC). HUVEC exposed to BXPC3 conditioned medium, rTF, MP-R, PPP-R high or low were not capable to enhance TG as compared to the control. Only HUVEC exposed to BXPC3-dMPs displayed a high amount of TF (563±47 pg/ml), whereas HUVEC exposed to all other experimental conditions did not express any detectable TF.

Figure 1. Thrombogram parameters in normal PPP of HUVEC cells exposed or not (control) to respectively BXPC3 derived vesicles (BXPC3-dEVs), BXPC3 conditioned medium depleted in vesicles (BXPC3-MC), human recombinant TF Dade Innovin (5nM TF, phospholipids and calcium), PPP- Reagent High (5pM TF and 4µM phospholipids), PPP-Reagent Low (1pM TF and 4µM phospholipids) or MP-Reagent (no TF and 4µM of phospholipids).
Values are mean ± sd of 3 experiments.



	HUVEC control	HUVEC + BXPC3-dEVs	BXPC3-MC	HUVEC + BXPC3-MC	HUVEC + Innovin	HUVEC + PPP-R High	HUVEC + PPP-R Low	HUVEC + MP-R
TF (pg/ml)	0,00	563,84 ± 47,47	361,1 ± 47,49	0,00	0,00	0,00	0,00	0,00

Table I. *Tissue factor concentration of HUVEC cells exposed or not (control) to respectively BXPC3 derived vesicles (BXPC3-dEVs), BXPC3 conditioned medium depleted in vesicles (BXPC3-MC), human recombinant TF Dade Innovin, PPP-Reagent High, PPP-Reagent Low or MP-Reagent. Values are mean ± sd of 3 experiments.*

Conclusion

CaCe-dMPs induce a procoagulant shift of EC characterised by marked expression of TF and enhancement of TG. These properties are transferred to following generations. TF alone is not sufficient to induce the procoagulant shift of EC. The ensemble of CaCe-dMP expressing TF is the vector of the procoagulant transformation of cancer cells. This property of CaCe-dMPs could lead to new therapeutic targets for the prevention of CAT.