TCTP in Extracellular Vehicles

Dangerous Cargo?

TCTP in extracellular vehicles: dangerous cargo?

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Endothelial cells line all blood vessels and are critical for vascular homeostasis. Intercellular communication is essential for fulfilling their function. Classically, cells communicate via direct cell-cell-contact or over long distances via soluble factors such as cytokines and hormones. In addition, transfer of information via extracellular vesicles (EVs) has emerged as a third mechanism of communication [1].

Extracellular vesicles (EVs) are secreted plasma membrane structures comprising exosomes, microvesicles and apoptotic bodies [2]. The potential of information transfer via EVs reaches beyond the possibilities of classical communication, due to their ability to enclose, or even expose, a variety of biomolecules. Cargos of EVs include miRNA, DNA, mRNA, lipid, and protein components, even active enzymes. Upon contact with target cells, the EV cargo can influence the recipient cell's gene expression and cellular phenotype [1].

Activation or stress can trigger the release of EVs from endothelial cells. In many diseases, including cancer or atherosclerosis, endothelial injury is a crucial and sometimes initial event. Indeed, increased circulating levels of EVs have been detected in patients with atherosclerosis [3, 4], and in cancer, endothelial-derived EVs have been shown to possess anti-apoptotic, pro-proliferative activity via their ability to transfer oncogenes [5-7].

Endothelial cell injury and endothelial cell apoptosis is currently considered a trigger event of pulmonary arterial hypertension, a fatal disease that is characterized by excessive remodeling of distal pulmonary arteries. So far, only limited studies suggest a role of EVs in PAH. Circulating microparticles have been detected in the blood of PAH patients [8-10] and mice developed pulmonary hypertension following treatment of EVs-derived from mice with established monocrotaline-induced PH[10]. In vitro exposure to hypoxia stimulated the release of EVs with the capacity to influence cellular behaviour, such as survival and proliferation [11]. So far, a mechanistic explanation on how EVs from endothelial cells can drive the aberrant proliferation of underlying smooth muscle cells has been lacking.

In the current issue of the journal, Ferrer and colleagues [12] shed light on this exciting question. The authors not only demonstrate that EVs could serve as a possible communication between endothelial cells and PASMC, but also identified their cargo, TCTP, as a mediator of vascular remodeling underlying PH. Translationally controlled tumor protein, TCTP, is considered an oncogene product, as it is overexpressed in various cancers and is associated with increased cell proliferation. Therefore, transfer of TCTP between endothelial cells and smooth-muscle cells could drive the vicious circle of vascular remodeling.

In a series of experiments, the authors presented that blood outgrowth endothelial cells (BOEC) not only produce EVs but that TNF-α, a pro-inflammatory cytokine, exaggerated the exosomal release. Inflammation and inflammatory mediators, such as TNF-α, are strongly implicated in disease pathogenesis by inducing cell injury [13]. The notion that TNF- α induced not only the release of EVs but rather a specific subset of EVs harboring the pro-proliferative protein TCTP further highlights the interplay between endothelial cell injury and smooth muscle cell remodeling.

Ferrer et al. [12] were able to identify exosomes as the EV-compartment with highest levels of TCTP by use of multiple markers as well as inclusion and exclusion criteria. Strengthening that finding, TCTP was detected within the multivesicular compartment in the cytoplasm of BOEC. This precise characterization can be seen as exemplary for other studies, as classification of EVs currently still presents a challenge in EV research. Isolation techniques such as ultracentrifugation generally co-isolate different types of EVs [14] and markers used for classification are sometimes not exclusive (such as CD63, which is present on both exosomes and microvesicles).

Using two complementary experiments, Ferrer et al. [12] elegantly show the EV-mediated transfer of TCTP from BOEC to PASMC. Fluorescent labelling of EVs allowed for tracking of EVs into recipient cells, and TCTP, as a cargo of the EVs, was detected in increased levels in PASMC. Nevertheless, the exclusive demonstration of EV transfer between cells in vivo and in vitro remains one of the biggest challenges. Therefore, these results should be interpreted with caution, as the possibility remains that at least some of the increased TCTP observed after EV exposure is due to EV-mediated signalling rather than direct transfer of the cargo.

Ferrer et al. [12] conclusively demonstrate the detrimental role of increased TCTP in recipient cells as both 1) incubation of PASMC with supernatants from umbilical endothelial cells overexpressing TCTP, and 2) direct overexpression of TCTP in PASMC led to enhanced proliferation of PASMC. These findings were further substantiated by determining the circulating levels of TCTP in the monocrotaline-rat model and human PAH patients. In both settings, the circulating levels of TCTP were increased, further pointing towards TCTP exerting its action in an exogenous fashion. While these findings cumulatively demonstrate that 1) TCTP is present in EVs from BOEC and 2) PASMC can take up TCTP-derived from EVs, it is still unclear whether TCTP transferred exclusively via EVs can exert the functional detrimental effects observed in PASMC or in a mechanism independent from EVs.

Other essential questions arising from these findings are: do IPAH patients possess elevated circulating EV harboring TCTP? If so, can EV-TCTP exert functional effects on target cells and do such EVs harbor additional biomarkers of relevance to IPAH?

Cumulatively, Ferrer et al. [12] present an exciting possible link between EC injury and the IPAH-specific malfunctional PASMC phenotype. They clearly identified the detrimental role of TCTP and provide first insight into the mechanistic role of EVs in IPAH. Transfer of proteins between cells via EVs is an important and advancing field of research, especially considering the therapeutic potential of these vesicles and their cargos.

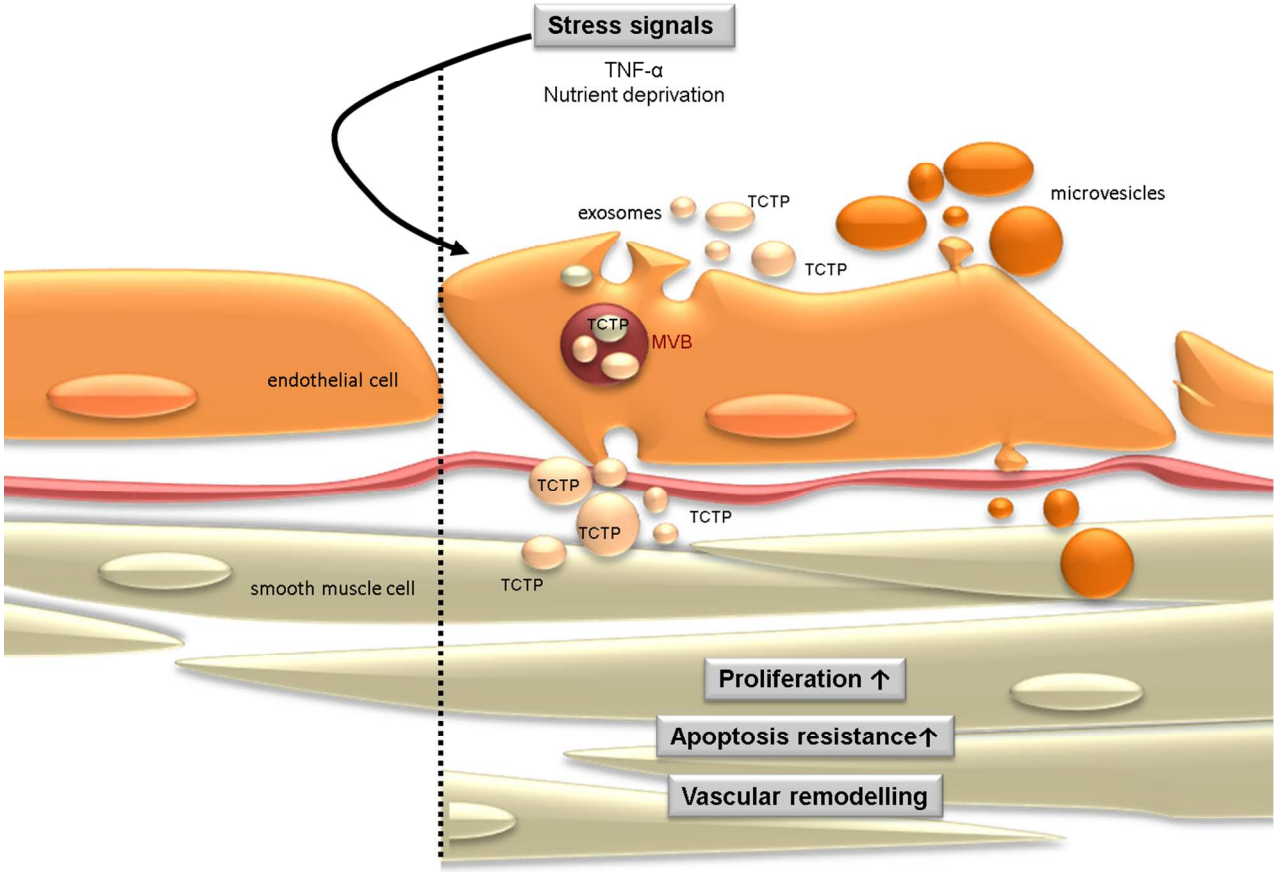

Figure 1) EV-derived TCTP in vascular remodeling. Upon stimulation, for example through injury induced by nutrient starvation or TNF-a, endothelial cells react with increased secretion of extracellular vesicles, mainly exosomes from endosomal origin and microvesicles, shed from the plasma membrane. TCTP, translationally controlled tumor protein, from cytoplasmic multivesicular bodies (MVB) is sequestered into exosomes and released from endothelial cells. Uptake of TCTP by PASMC induces aberrant proliferation, thereby driving vascular remodeling. TNF = tumor necrosis factor; MVB = multivesicular body; TCTP = translationally controlled tumor protein.

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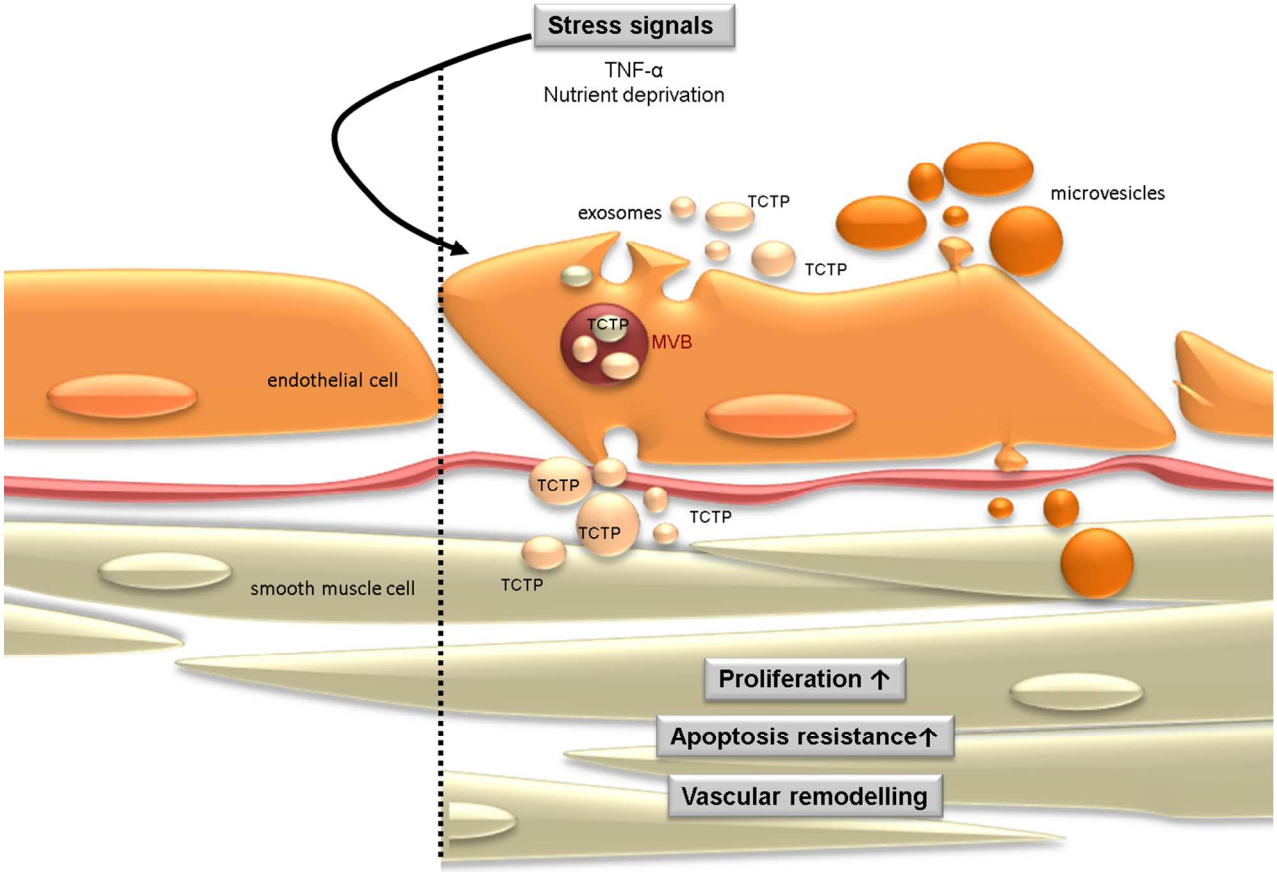

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