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Cytotoxicity, cytokine release and ER stress-autophagy gene expression in endothelial cells and alveolar-endothelial co-culture exposed to pristine and carboxylated multi-walled carbon nanotubes

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ABSTRACT

Recently we found that direct exposure of human umbilical vein endothelial cells (HUVECs) to multi-walled carbon nanotubes (MWCNTs) might induce toxicological responses through the modulation of ER stress gene expression, but whether this signal could be transferred from other cells to endothelial cells (ECs) is unknown. This study investigated the toxicity of pristine and carboxylated MWCNTs to HUVECs and alveolar-endothelial co-culture, the later of which could mimic the possible signaling communications between ECs and MWCNT exposed alveolar cells. The results showed that direct contact with high levels of MWCNTs induced cytotoxicity and modulated expression of genes associated with ER stress (*HSPA5*, *DDIT3* and *XBP-1s*) and autophagy (*BECN1* and *ATG12*) both in A549-THP-1 macrophages cultured in the upper chambers as well as HUVECs. However, most of these responses were minimal or negligible in HUVECs cultured in the lower chambers. Moreover, significantly increased cytokine release (interleukin-6 and soluble vascular cell adhesion molecule-1) was only observed in MWCNT exposed HUVECs ($p < 0.01$) but not HUVECs cultured in the lower chambers ($p > 0.05$). The minimal or even absent response was likely due to relatively low translocation of MWCNTs from upper chambers to lower chambers, whereas A549-macrophages cultured in the upper chambers internalized large amount MWCNTs. The results indicated that ER stress-autophagy signaling might not be able to transfer from alveolar cells to endothelial cells unless sufficient MWCNTs are translocated.

1. Introduction

Multi-walled carbon nanotubes (MWCNTs) are high aspect ratio engineered nanoparticles (NPs) with a high length to width ratio. Since their discovery, they already became one of the most popular carbon based NPs since they have many important applications, for instance electronics (Kuang et al., 2016), energy storage (De Volder et al., 2013), water treatment (Burakova et al., 2018) and biomedicine (Gong et al., 2013; Mocan et al., 2017). This could increase the exposure of human beings to MWCNTs, and there is a health concern about their potential toxicity. Indeed, extensive studies have already revealed that MWCNT exposure might promote pulmonary fibrosis (Sharma et al., 2016), oxidative damage (Moller et al., 2014), reproductive and developmental toxicity (Ema et al., 2016) as well as development of cardiovascular diseases (Moller et al., 2016). Still, the toxic effects of MWCNTs are largely unknown and more studies are needed to further investigate the molecular mechanisms.

Recently, it was shown that NP exposure might induce toxic effects through the modulation of ER stress response (Cao et al., 2017; Ou et al., 2016). ER is an organelle crucial for proper function of cells, and perturbation of the normal function of ER could lead to a condition termed as ER stress, which could finally regulate the death of cells (Cao and Kaufman, 2014; Logue et al., 2013). Interestingly, some recent studies revealed that exposure to NPs might modulate the ER stress response not only at the places of contact, but also in systemic organs. For example, (Huo et al., 2015) found that intratracheal instillation of Ag NPs to mice promoted a strong ER stress response not only in lungs, but also in livers and kidneys. Similarly, (Yang et al., 2015) also found a strong ER stress in livers of mice gavaged to ZnO NPs, which is associated with NP induced liver damage. However, it remains unclear if the activation of ER stress in systemic organs is due to the direct effects of NPs accumulated in systemic organs, or the indirect effects of

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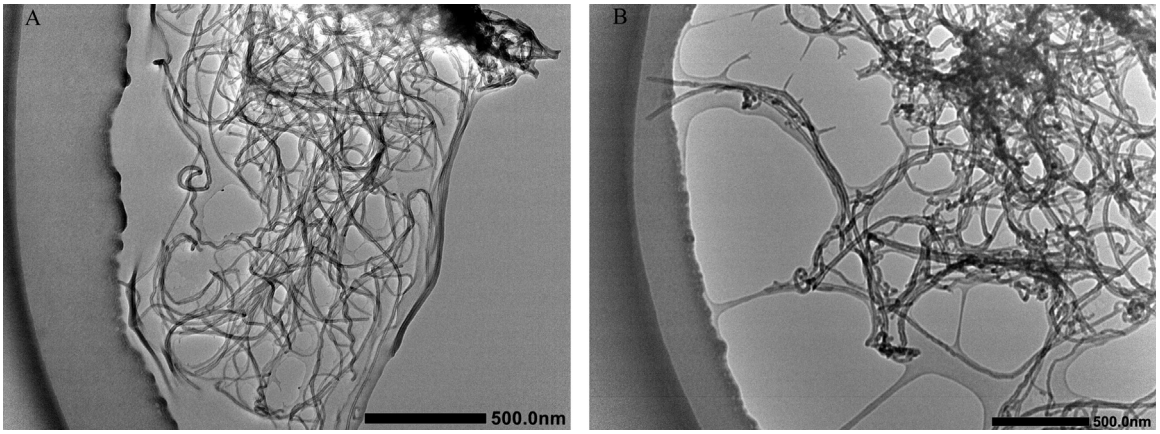

Fig. 1. The representative TEM images of pristine (code XFM19; A) and carboxylated MWCNTs (code XFM21; B).

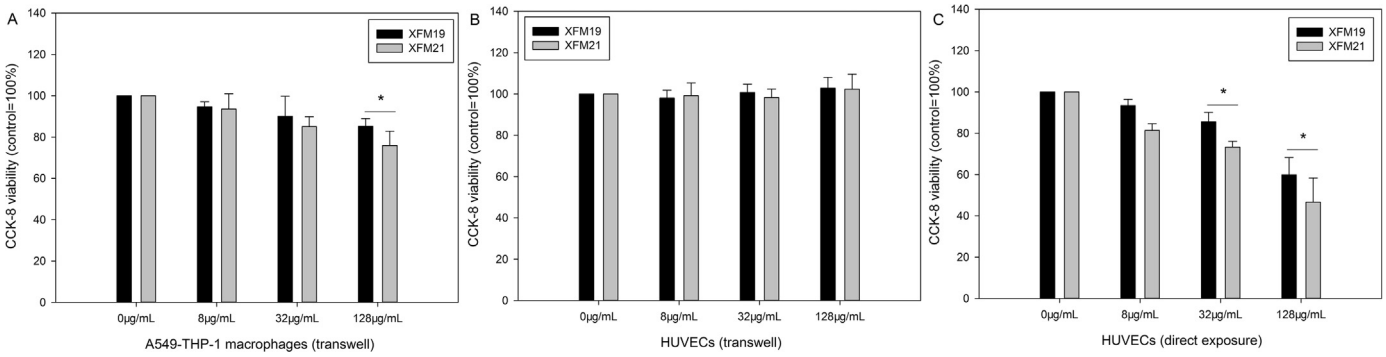

Fig. 2. The cytotoxicity of pristine (code XFM19) and carboxylated MWCNTs (code XFM21) as assessed by CCK-8 assay. Various concentrations of MWCNTs were apically applied to transwells or directly applied to HUVECs. After 24 h exposure, CCK-8 assay was used to indicate cytotoxicity to A549-THP-1 macrophages cultured in upper chambers (A), HUVECs cultured in lower chambers (B) or HUVECs directly exposed to MWCNTs (C). *, $p < 0.05$, compared with control.

signaling communications between places of contact and systemic organs.

We have recently shown that MWCNT exposure modulated gene expression associated with ER stress in human umbilical vein endothelial cells (HUVECs), which could be responsible for MWCNT induced cytotoxicity and endothelial activation (Long et al., 2017). This suggested that direct contact with MWCNTs might influence ER stress in endothelial cells (ECs), but whether ER stress signaling could be transferred from MWCNT exposed cells to ECs is unknown. In this study, we investigated the toxicity of pristine and carboxylated MWCNTs to both HUVECs and alveolar endothelial co-culture, the later of which could mimic the endothelial activation following inhalational MWCNT exposure. The alveolar-endothelial co-culture was developed by using transwells with 0.4 μM pore sizes, because they have very limited translocation rate and could thus be used to study the possible signaling communications between alveolar and endothelial cells (Cao et al., 2016, 2015; Klein et al., 2013). Transmission electron microscopy (TEM) was used to visualize the internalization and translocation of MWCNTs. Cytotoxicity, release of inflammatory cytokines and expression of ER stress were investigated in both models after MWCNT exposure. In addition, the expression of typical autophagy genes was also measured in both models. Autophagy is a classical degradation pathway for intracellular pathogens as well as damaged organelles and proteins, and convincing data showed that non-degradable NPs could induce dysfunction of autophagy, leading to autophagic cell death (Ou et al., 2016; Soenen et al., 2015). It has been suggested that ER stress and autophagy are dynamically interconnected, that ER stress can either stimulate or inhibit autophagy (Rashid et al., 2015).

2. Materials and methods

2.1. Cell cultures

A549 lung epithelial cells (ATCC), THP-1 monocytes (ATCC) and HUVECs (ScienCell Research Laboratories; Carlsbad, CA, USA) were cultured in supplemented DMEM/F-12 medium, DMEM/high glucose medium (Hyclone, GE Healthcare) and endothelial cell medium (ECM; ScienCell Research Laboratories; Carlsbad, CA, USA), respectively, as we previously described (He et al., 2017; Ji et al., 2017; Jiang et al., 2016). THP-1 cells were differentiated into macrophages by the treatment of phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, MO, USA) as we described earlier (Jiang et al., 2016). For the development of alveolar-endothelial co-culture, A549 cells were seeded at a density of 5×10^3 per well in 24-well format transwells (0.2% gelatin pre-coated; cell culture area 0.33 cm^2, diameter of pores 0.4 μm, transparent polycarbonate membrane) or 5×10^4 per well in 6-well format transwells (0.2% gelatin pre-coated; cell culture area 4.67 cm^2, diameter of pores 0.4 μm, transparent polycarbonate membrane; Corning Costar, Kennebunk, ME, USA). On day 5, the THP-1 macrophages were seeded at a density of 5×10^3 per well in 24-well format transwells or 5×10^4 per well in 6-well format transwells, and HUVECs were seeded at a density of 4×10^4 per well in 24-well plates or 2×10^5 per well in 6-well plates (in separate plates). On day 7, the A549 cells, THP-1 macrophages and HUVECs were co-cultured and then apically exposed to MWCNTs. The transwells were incubated with 0.3 mL DMEM/F-12 medium in upper chamber and 0.7 mL ECM in lower chamber (24-well format) or 3 mL DMEM/F-12 medium in upper chamber and 3 mL ECM in lower chamber (6-well format), with cell culture medium changed every 2–3 days from both chambers. For experiments in HUVECs, cells