News Release

Title

Ferroptosis-dependent extracellular vesicles from macrophage contribute to asbestos induced mesothelial carcinogenesis through loading ferritin

Key Points

○Asbestos triggers FedEVs-secretion from macrophages under ferroptotic process.

○Extracellular vesicles transport asbestos-derived mutagenic factor to mesothelial cells.

○Major mutagenic factor transported by extracellular vesicles is ferritin

○Mesothelial cells receiving EVs were at the S and G2/M phases with increased iron-dependent DNA damage.

Summary

Asbestos-associated diseases remain a social burden worldwide. Our previous studies identified asbestos-induced iron-rich milieu for mesothelial cells with ceaseless macrophage ferroptosis. However, molecular mechanisms how this mutagenic milieu influences mesothelial cells have not been elucidated yet. Here, we propose a novel mechanism that extracellular vesicles (EVs) mediate asbestos-associated mutagenic factors to mesothelial cells. In a mice model of intraperitoneal crocidolite injection, mutagenic milieu highly expressed CD63, an exosomal marker. We then used a GFP-CD63 labeled THP-1 macrophage model exposed to crocidolite/iron, which generated EVs under ferroptotic process. We observed that MeT-5A mesothelial cells can receive and internalize these EVs. Furthermore, we comprehensively analyzed the ferroptosis-dependent EVs (FedEVs) for transported proteins and identified ferritin heavy/light chains as major components. Therefore, we inferred that FedEVs transport iron from ferroptotic macrophages to mesothelial cells. RNA sequencing revealed that the mesothelial cells receiving higher amounts of the FedEVs were mitotic, especially at the S and G2/M phases, by the use of Fucci mesothelial cells. Nuclear 8-hydroxy-2`-deoxyguanosine and γ-H2AX were significantly increased in the recipient mesothelial cells after exposure to FedEVs. Collectively, we here demonstrate a novel mechanism that FedEVs act as a key mutagenic mediator by transporting iron, which contributes to asbestos-induced mesothelial carcinogenesis.

Research Background

Asbestos-associated carcinogenesis has been studied from two distinct standpoints, direct and/or indirect effects. Direct effects suggest that asbestos fibers phagocytosed by mesothelial cells, reach inside the nucleus and generate mutations almost physically. On the other hand, indirect effects hypothesize that asbestos localizes inside macrophages, and the oxidative stress based on frustrated phagocytosis induces mesothelial genetic alteration. Our previous study identified the asbestos-induced carcinogenic milieu that the asbestos fibers are located within macrophages and not in

mesothelial cells whereas DNA damage in mesothelial cells was increased close to the asbestos-induced mutagenic milieu. However, little is known on the molecular mechanisms how this mutagenic milieu influences mesothelial cells toward carcinogenesis. Thus, we hypothesized that ferroptosis-dependent EVs (FedEVs) may mediate iron and mutagenic factors, as a novel mechanism of mutagenic niche.

Research Results

We first performed histological evaluation, using a mouse model of ip crocidolite injection to elucidate the tissue localization of FedEVs in the asbestos-induced granuloma. Immunofluorescence analysis revealed that the asbestos fibers were inside CD68-positive macrophages and neither in the α-SMA-positive myofibroblasts nor mesothelin-positive mesothelial cells. Of note, CD63, a marker for EVs, were abundant with a granular pattern in mesothelial cells and myofibroblast cells in proximity to the granuloma (Figure 1ab). We next studied the possibility of FedEV uptake by the mesothelial cells. We established the THP1 and HT1080 cells, stably expressing *GFP-CD63*, as FedEV donor cells. After addition of GFP-labeled FedEVs to MeT-5A mesothelial cells, GFP-CD63 originating from donor cells were observed on the plasma membrane of recipient MeT-5A cells, starting from 0.5 h, which continued till 3 h with the formation of bleb-like structures. FedEVs were completely internalized thereafter by the recipient mesothelial cells at 6 to 9 h, as seen by the coexistence of GFP-CD63 and endogenous CD63 in the cytoplasm (endosome~late endosome~lysosome) evaluated by the *z*-axis (Figure 1c).

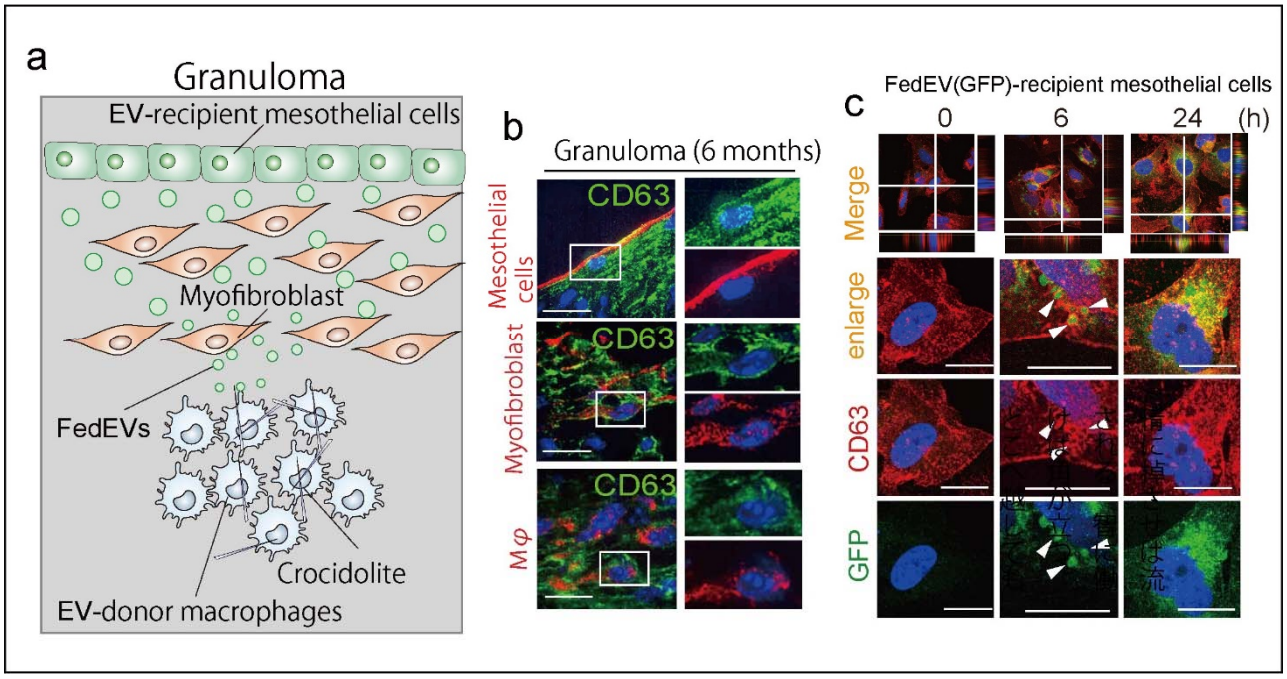

Figure 1: CD63-positive ferroptosis-dependent EVs (FedEVs) derived from macrophages simultaneously exposed to asbestos and iron. (a) Schematic image of all the components surrounding

the asbestos-induced granuloma. (b) High-resolution fluorescent image in granuloma; red, cell-specific marker of each cell-type (mesothelin, αSMA and CD68, respectively); green, CD63; blue, Hoechst33342 nuclear staining (bar = 20 μm). (c) Fluorescent cellular images of recipient mesothelial cells phagocytosing GFP-positive FedEVs derived from THP1 macrophage cells exposed to asbestos; red, endogenous CD63; bar = 10 μm).

To comprehensively identify the protein components in FedEVs, we performed a proteomic analysis using LC-MS. We integrated differentially expressed proteins (non-treated *vs* crocidolite and Fe) as proteomap images, accounting for the entire items and listed the top 15 differentially increased proteins. Ferritin light and heavy chains (FTL and FTH) were the proteins, identified to be associated with iron storage in the FedEVs. Of note, only FedEVs from iron- or iron and crocidolite-treated groups contained a high amount of FtL and FtH, which was associated with iron excess (Figure 2a). To evaluate the DNA damage in the FedEVs recipient mesothelial cells, we performed the FACS and imaging analysis of oxidative DNA base modification and DNA double-strand breaks (Figure 2b). 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most abundant DNA modifications caused either by hydroxyl radical, single oxygen or peroxynitrite. γH2AX is rapidly recruited to the DNA double-strand breaks for repair, thus working as a DNA damage marker. Mesothelial cells were exposed to crocidolite-derived FedEVs from THP1 cells, which were evaluated 24 h afterwards. We observed the increase both in nuclear 8-OHdG and γH2AX in the recipient cells of FedEVs

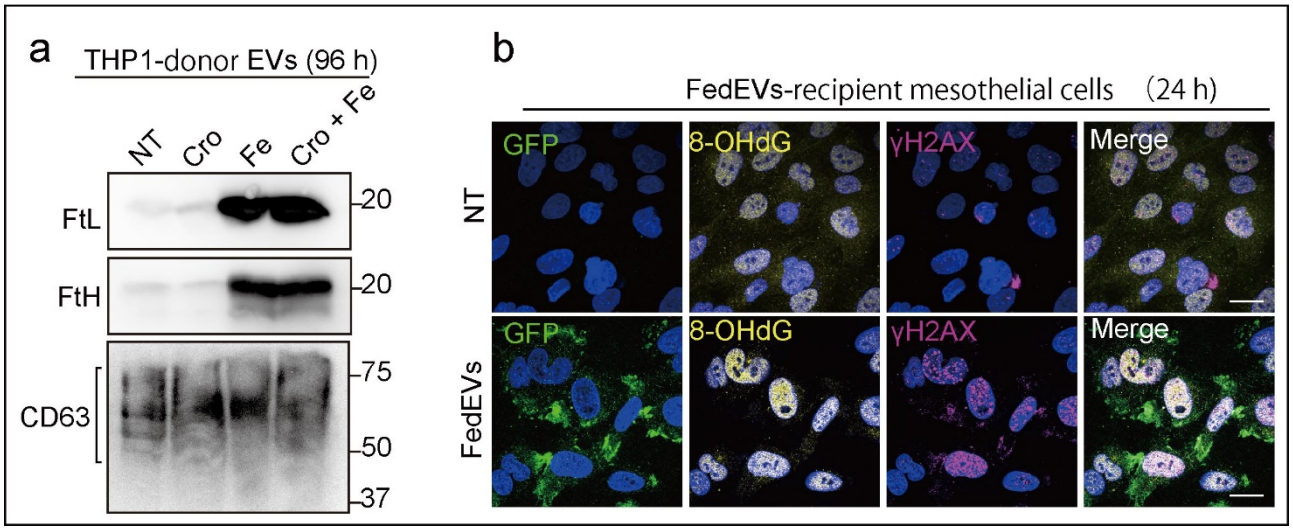

Figure 2: Phagocytosis of FedEVs causes oxidative DNA base modification (8-hydroxy-2′-deoxyguanosine, 8-OHdG) and DNA double-strand breaks detected by γH2AX in the recipient mesothelial cells. (a) Immunoblot analysis of FedEVs from THP1 macrophage. (b) Immunofluorescent images of mesothelial cells after exposure of THP1-derived FedEVs for 24 h;

green, CD63-positive FedEVs from THP1 cells; yellow, 8-OHdG; magenda, γH2AX with blue Hoechst33342 nuclear staining (bar = 10 μm).

Publication

Fumiya Ito, Katsuhiro Kato, Izumi Yanatori, Toyoaki Murohara and Shinya Toyokuni. Ferroptosis-dependent extracellular vesicles from macrophage contribute to asbestos-induced mesothelial carcinogenesis through loading ferritin. Redox Biol. 2021 Oct 21;47:102174. doi: 10.1016/j.redox.2021.102174. Online ahead of print. PMID: 34700146

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