News Release

Title

Asbestos conceives Fe(II)-dependent mutagenic stromal milieu through ceaseless macrophage ferroptosis and β-catenin induction in mesothelium

Key Points

○Asbestos is a carcinogen causing malignant mesothelioma.

○Asbestos associated carcinogenesis has not been elucidated yet. This report clarified those mechanisms.

○Phagocytosis of asbestos by macrophages results in their distinctive necrotic death; initially lysosome-depenent cell death and later ferroptosis, which increase intra- and extra-cellular catalytic Fe(II).

○β-catenin overexpression in mesothelial cells induced higher intracellular catalytic Fe(II)

○ Mesothelial cells after challenge of H_2O_2 under β-catenin overexpression presented low *p16*^{INK4A} expression with a high incidence of deletion in *p16*^{INK4A} locus.

Summary

Asbestos is still a social burden worldwide as a carcinogen causing malignant mesothelioma. Whereas recent studies suggest that local iron reduction is a preventive strategy against carcinogenesis, little is known regarding the cellular and molecular mechanisms surrounding excess iron. Here by differentially using high- risk and low-risk asbestos fibers (crocidolite and anthophyllite, respectively), we identified asbestos-induced mutagenic milieu for mesothelial cells. Rat and cell experiments revealed that phagocytosis of asbestos by macrophages results in their distinctive necrotic death; initially lysosome-depenent cell death and later ferroptosis, which increase intra- and extra-cellular catalytic Fe(II). DNA damage in mesothelial cells, as assessed by 8-hydroxy-2'-deoxyguanosine and γH2AX, increased after crocidolite exposure during regeneration accompanied by β-catenin activation. Conversely, β-catenin overexpression in mesothelial cells induced higher intracellular catalytic Fe(II) with increased G2/M cell-cycle fraction, when *p16*^{INK4A} genomic loci localized more peripherally in the nucleus. Mesothelial cells after challenge of H_2O_2 under β-catenin overexpression presented low *p16*^{INK4A} expression with a high incidence of deletion in *p16*^{INK4A} locus. Thus, crocidolite generated catalytic Fe(II)-rich

mutagenic environment for mesothelial cells by necrotizing macrophages with lysosomal cell death and ferroptosis. These results suggest novel molecular strategies to prevent mesothelial carcinogenesis after asbestos exposure.

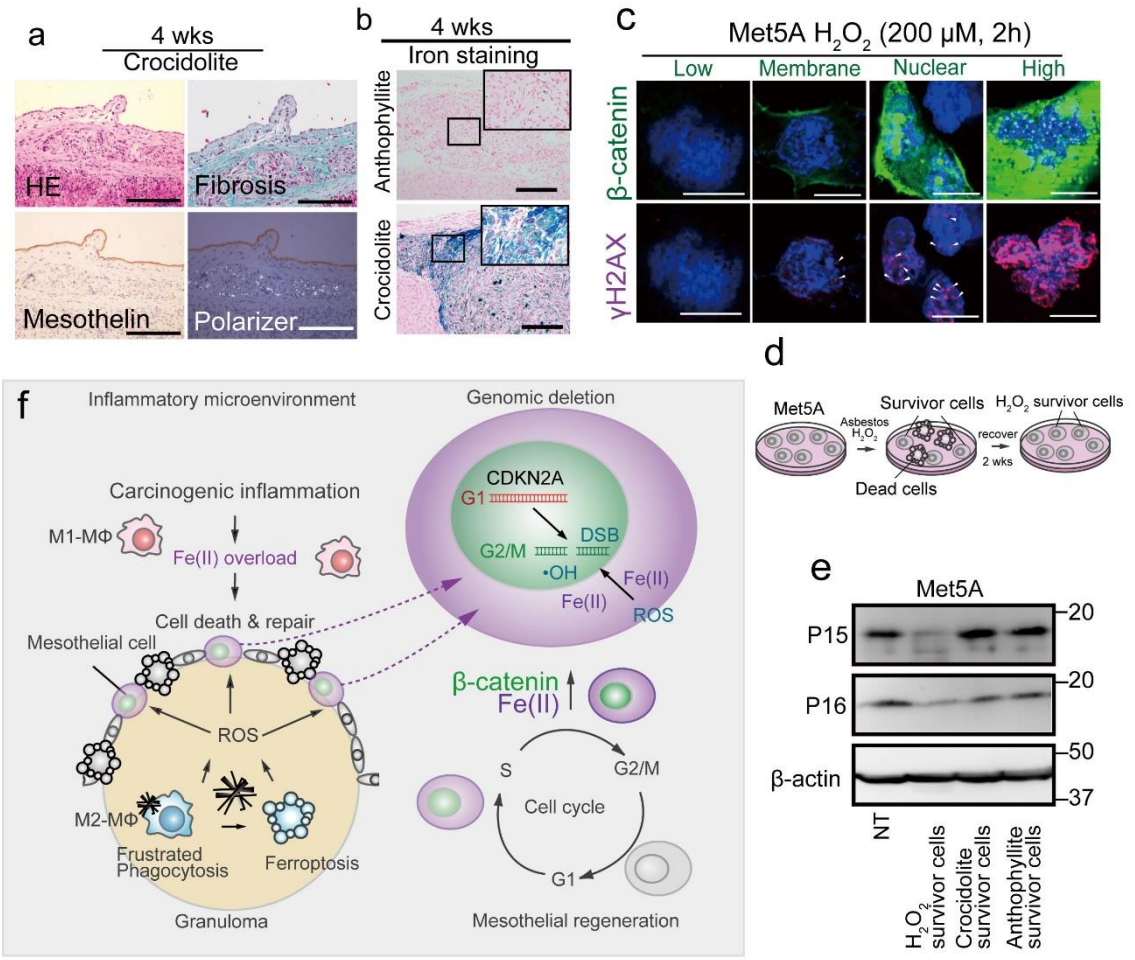
Research Background

Asbestos is still a social burden worldwide as a carcinogen causing malignant mesothelioma. Asbestos-associated carcinogenesis has been studied from two distinct standpoints, direct and/or indirect effects. Direct effects suggest that asbestos fibers are phagocytosed by mesothelial cells, reach inside the nucleus. On the other hand, indirect effects hypothesize that asbestos fibers localize inside macrophages and that oxidative stress from frustrated phagocytosis induces mesothelial genetic alterations. The question is not yet settled. Pathogenicity of asbestos fibers has been associated with iron, as exemplified by the asbestos body in histology. Intraperitoneal injection of ferric saccharate induces MM in *wild-type* rats. Regarding the mutation spectrum collected using human samples, ~70% of MM exhibits $p16^{INK4A}$ homozygous deletion Here we for the first time demonstrate that ceaseless macrophage necrotic death, through lysosomal cell death and ferroptosis, generates catalytic Fe(II)-rich stromal mutagenic microenvironment for mesothelial cells and further that the associated activation of β-catenin is advantageous in mesothelial cells to obtain deletion of $p16^{INK4A}$ via increased fraction of G2/M phase, increased intracellular catalytic Fe(II) and juxta-nuclear membrane position of $p16^{INK4A}$ genomic loci.

Research Results

To elucidate the cellular mechanism toward iron-rich stromal microenvironment, we first performed the histological analysis in rats injected intraperitoneally either with high- or low-risk asbestos fibers. Four weeks after the asbestos injection, asbestos fibers were inside macrophages and not in mesothelial cells (a). Intra-granuloma macrophages significantly accumulated iron only after crocidolite exposure (b). Peritoneal macrophages collected iron most among all the inflammatory cells, and catalytic Fe(II) was significantly increased intracellularly and extracellularly only after crocidolite exposure. We next examined the biological responses of mesothelial cells after crocidolite exposure and we discovered the association of Wnt/β-catenin pathway with mesothelial regeneration. γH2AX levels (DNA double strand break marker) further increased in β-catenin-overexpressed mesothelial cells in the

presence of iron and H_2O_2(c). Iron- and H_2O_2-rich environment decreased both p15 and p16 expression in mesothelial cells, and the $p16^{Low}$ mesothelial cells survived in the environment (d, e). We found that $p16^{INK4A}$ loci were localized to central nuclei at G1 phase whereas the localization was altered to the near nuclear membrane at G2/M phase.



Figure

(a) Peritoneal histology of rat model 4 wks after intraperitoneal injection of crocidolite, a high-risk asbestos; HE, hematoxylin & eosin staining; Masson trichrome staining; polarizer detects asbestos fibers (bar = 50･m). (b) Iron deposition by Berlin blue staining is significantly higher after crocidolite exposure than anthophyllite exposure. (c) Association of β-catenin expression with γH2AX in Met5A cells under exposure to H_2O_2 (200 μM, 2 h; green, β-catenin-GFP; magenta, γH2AX; bar = 5 μm). (d, e) Scheme of culture system for mesothelial survivor

analysis using Met5A or LP-9 human mesothelial cells. Immunoblot analysis in Met5A and LP9 cells for $p15^{INK4B}$ and $p16^{INK4A}$ after exposure of H_2O_2 (25 μM), asbestos (25 μg/cm^2) for 3 days. After 2 weeks of recovery, survived cells were used for protein detection analysis. (f) Graphical abstract illustrating the key concepts on the iron-rich somatic mutagenic milieu after exposure to crocidolite (left). Mesothelial regeneration after exposure to crocidolite accompanies β-catenin overexpression, which increases the risk of $p16^{INK4A}$ deletion via multiple mechanisms, such as increased G2/M phase fraction, higher intracellular catalytic Fe(II) and peripheral transfer of $p16^{INK4A}$ loci in nucleus

Research Summary and Future Perspective

In conclusion, our findings highlight the importance of catalytic Fe(II)-dependent mutagenic stromal milieu in mesothelial genomic alterations in that asbestos-induced indirect damage affects regenerative mesothelia with β-catenin-dependence. We discovered that mesothelial activation of β-catenin pathway during regeneration brings multiple advantages for their $p16^{INK4A}$ deletion. Our results would be helpful for establishing strategies to prevent MM in people already exposed to asbestos. In addition to iron modulation and anti-inflammation as already suggested, β-catenin pathway would be another molecular target to prevent mesothelial carcinogenesis.

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Publication

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