主論文の要旨

Cancer-promoting role of adipocytes in asbestos-induced mesothelial carcinogenesis through dysregulated adipocytokine production

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<Background>

Adipose tissue, previously known only for its role in lipid storage and energy metabolism, is now recognized as an important endocrine organ which secretes various biologically active peptides collectively known as the adipocytokines. Examples include leptin, adiponectin, MCP-1, IL-6, PAI-1 and adiponectin. Most of these adipocytokines are pro-inflammatory but some are anti-inflammatory such as adiponectin. The role of adipose tissue in promoting inflammation has been established by many studies and in addition, epidemiological studies suggested a correlation between obesity and higher risk of cancer.

Our study was aimed to investigate the cancer promoting role of adipose tissue in asbestos-induced mesothelial carcinogenesis. Previous data from animal studies showed a higher frequency of malignant mesothelioma (MM) development following intraperitoneal injection of asbestos fibers compared to intrapleural injection. Adipose tissue is abundant in the peritoneal cavity and is also present at a lesser extent in the pleural cavity such as in the submesothelial space of parietal pleura. We hypothesized that the high amount of adipose tissue in the peritoneal cavity correlates with the higher frequency of peritoneal MM following asbestos injection and the endocrine activity of adipose tissue might play a role in promoting mesothelial carcinogenesis.

<Materials and methods>

Three different types of asbestos fibers were used in our study: chrysotile, crocidolite and amosite fibers. For in vitro study, we used mouse 3T3-L1 preadipocyte cell line. 3T3-L1 preadipocytes were induced to differentiate into mature adipocytes followed by exposure to asbestos fibers for 72 hours. Different analyses were then performed on the adipocytes such as microarray gene expression analysis, RT-PCR, ELISA, etc. For animal study, asbestos fibers were injected intraperitoneally into male DDY mice and epididymal fat pads were then harvested from the mice after 3 days and used for different analyses.

<Results>

Maturation of adipocyte from 3T3-L1 preadipocyte was confirmed by oil red O staining, which stains for lipid droplets (Fig. 1A). We observed uptake of asbestos fibers by the mature adipocytes using both light microscopy and transmission electron microscopy (Fig. 1B). At higher magnification, vesicular membranes surrounding asbestos fibers could be seen. As asbestos fibers are known to be cytotoxic to both mesothelial cells and macrophages, we examined the cytotoxic effect on adipocytes and found that asbestos fibers did not induce

adipocyte cell death (Fig. 1C).

Changes in gene expression of asbestos-treated adipocytes were assessed using microarray analysis and we found upregulation of some inflammation related genes, e.g. serum amyloid A3, haptoglobin, urokinase-type plasminogen activator (Table I). Moreover, expression levels of adipocytokines such as MCP-1 (also known as Ccl2) and Prl2c5 were also upregulated. Gene expression alterations were found to occur at a lesser extent in amosite-treated adipocytes. Results of microarray were confirmed by real-time RT-PCR (Fig. 2A). Assessment of other adipocytokines (PAI-1, leptin, IL-6, adiponectin) revealed altered expression levels as well. Increased MCP-1 secretion into the culture medium by asbestos-treated adipocytes was confirmed by ELISA (Fig. 2B).

Adipose tissue from asbestos-injected animals also showed increased mRNA level of MCP-1 and Prl2c5, but changes for other adipocytokines were not so consistent with the cultured adipocytes (Fig. 3A). Immunohistochemical staining of adipose tissue showed a higher MCP-1 protein level compared to control group (Fig. 3B). We also found a reduction in adipocyte size from asbestos-treated group (Fig. 3C).

MCP-1 is known as a potent chemoattractant for macrophages. We collected conditioned media from asbestos-treated adipocytes and measured the ability of the conditioned media to induce macrophage migration. Conditioned media from asbestos-treated adipocytes showed a greater ability to attract macrophages (Fig. 4). We also assayed the effects of recombinant MCP-1 on cell proliferation and migration. MCP-1 showed a marginal growth stimulatory effect on normal human mesothelial cell line, MeT-5A (Fig. 5A) but did not promote its migration (Fig. 5B). MCP-1 promoted the migration of human mesothelioma cells, Y-MESO-8A and Y-MESO-8D (Fig. 5B).

<Discussion>

Our study demonstrated for the first time that adipocytes were able to internalize particles such as asbestos fibers. Our findings corroborated those of others which suggested that adipocytes can perform phagocytosis in a macrophage-like manner. Moreover, we found that adipocytes responded to asbestos exposure by altering gene expressions including upregulation of genes related to inflammation (e.g. serum amyloid A3, haptoglobin, urokinase-type plasminogen activator). More importantly, adipocytes also upregulated expression of adipocytokines such as MCP-1 and Prl2c5, indicating that the endocrine activity of adipocytes can be altered by asbestos exposure.

Reduced adipocyte size observed in asbestos-injected mice might be caused by

this increase in metabolic rate. It is still unknown whether asbestos fibers can directly activate cell signaling pathways in adipocytes or the effects are mediated primarily by ROS produced in association with asbestos exposure.

Among the adipocytokines examined in our study, MCP-1 was consistently upregulated at both mRNA and protein levels after asbestos exposure. Moreover, we showed that conditioned media from asbestos-treated adipocytes were able to induce a higher macrophage migration, which is most probably associated with increased MCP-1 secretion. In addition, we showed that MCP-1 promoted mesothelial and mesothelioma cell growth and migration. Secretion of MCP-1 by the adipose tissue might thus play an important role in mesothelial carcinogenesis by recruiting more macrophages as well as promoting mesothelial cell proliferation and migration. On the other hand, the expression level of an anti-inflammatory adipocytokine, adiponectin, was suppressed, suggesting that the adipocytes might support an inflammatory environment by increasing production of pro-inflammatory adipocytokines and suppressing anti-inflammatory adipocytokine production.

<Conclusion>

Our results suggested a potential cancer-promoting role of adipocytes in asbestos-induced mesothelial carcinogenesis through dysregulated adipocytokine production. Dysregulated adipose endocrine activity by asbestos fibers disrupts the homeostatic state of tissue environment by favoring a pro-inflammatory condition. These adipocykines can thus represent a target to ameliorate inflammation following asbestos exposure.

Abbreviations: MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1; Prl2c5, prolactin family 2, subfamily c, member 5.