HEALTH EFFECTS INSTITUTE

STATEMENT

Synopsis of Research Report 182

Synergistic Effects of Particulate Matter and Substrate Stiffness on Epithelial-to-Mesenchymal Transition

BACKGROUND

Exposure to particulate matter (PM) from combustion sources has been associated with lung inflammation and injury, which trigger repair responses to restore normal tissue function. Dysregulation of these responses can result, over time, in fibrotic changes characterized by increased numbers of fibroblasts and myofibroblasts and abnormal deposition of collagen and fibronectin in the extracellular matrix, with consequent increases in matrix stiffness and impairment of gas exchange. One theory proposes that the fibroblasts are derived from alveolar epithelial cells that differentiate into mesenchymal cells. This process is referred to as epithelial-to-mesenchymal transition (EMT). Fibrosis in the lung can be progressive and fatal, as in idiopathic fibrosis, and is also a feature of chronic pulmonary diseases such as asthma, and it is thus important to understand profibrotic processes.

In the current study Dr. Thomas H. Barker, who was a recipient of HEI's Walter A. Rosenblith New Investigator Award, and his colleagues tested the hypotheses that alveolar epithelial cells grown on fibronectin substrates of increasing stiffness would undergo EMT and that the addition of fine PM (PM $\leq$ 2.5 µm in aerodynamic diameter [$PM_{2.5}$]) would enhance these effects.

APPROACH

These hypotheses were tested in cells grown in vitro using a combination of mechanical and biologic approaches. The investigators addressed the following specific aims: (1) determine the effects of substrate stiffnesses that ranged from values seen in healthy tissue to those seen in fibrotic tissue on lung-epithelial-cell contractility, induction of EMT, and activation of transforming growth factor beta (TGF-β), a key factor in the development of fibrosis, and (2) determine whether exposure to $PM_{2.5}$ exacerbates the effects of substrate stiffness on EMT and cell contractility.

The substrates on which the cells were grown consisted of polyacrylamide–bisacrylamide gels cross-linked to fibronectin. Various concentrations of bisacrylamide were used to achieve increasing degrees of stiffness. After allowing the cells to grow

What This Study Adds

- Barker and colleagues developed a useful and novel in vitro cell model to study the interaction between extracellular matrix stiffness and the transition of lung epithelial cells to mesenchymal cells — a process that could lead to fibrosis — and the potential effects of PM on this process.
- The results showed that ATII epithelial cells transitioned to mesenchymal cells on substrates of greater stiffness, as documented by loss of cell circularity, changes in the expression of E-cadherin and α-SMA, and increased activation of TGF-β and that the addition of ambient PM enhanced the effect of substrate stiffness on EMT and TGF-β activation, as compared with non-exposed cells.
- This study highlights the potential importance of cell–matrix interactions when evaluating the effects of environmental triggers, but more work will be needed to understand how PM might affect such interactions and to determine whether the mechanisms are relevant to in vivo processes.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Thomas H. Barker at the Georgia Institute of Technology and Emory University, Atlanta, Georgia, and colleagues. The complete report, *Synergistic Effects of Particulate Matter and Substrate Stiffness on Epithelial-to-Mesenchymal Transition*, (© 2014 Health Effects Institute), can be obtained from HEI or our Web site (see next page).

on the substrates for five days, Barker and colleagues assessed EMT through changes in cell shape, cell contractility (measured as stiffness), and expression of the cellular protein E-cadherin, a marker of epithelial cells, and alpha smooth muscle actin (α-SMA), a marker of mesenchymal cells. They also measured activation of TGF-β. To study the effects of ambient PM on EMT, the investigators grew the cells in the presence of resuspended $PM_{2.5}$ previously collected on filters in areas near roads in Atlanta.

RESULTS AND INTERPRETATION

Cultured alveolar type II (ATII) rat epithelial cells transitioned to mesenchymal cells on substrates of increased stiffness, as documented by loss of cell circularity, decreased expression of E-cadherin, and increased expression of α-SMA. Increased substrate stiffness was associated with increased cell stiffness and increased activation of TGF-β. The role of TGF-β was further demonstrated in an experiment in which ATII cells were grown on a soft substrate in media containing various concentrations of active TGF-β for two or five days. Exposure to TGF-β for five days was associated with decreased cell circularity, changes in surface markers, and increased cell stiffness.

Addition of ambient $PM_{2.5}$ at the highest concentration (corresponding to 10 $\mu g/cm^2$) increased EMT as measured by reduced cell circularity, decreased expression of E-cadherin, and increased expression of α-SMA, as compared with the responses in non-exposed cells at the same substrate stiffness. Cell stiffness also increased after exposure to $PM_{2.5}$, as compared with the non-exposed cells. In addition, ATII cells showed greater TGF-β activation with increasing substrate stiffness when exposed to $PM_{2.5}$ compared with the non-exposed cells.

In its independent review of the study, the HEI Health Review Committee noted that this was a carefully performed study with interesting mechanistic observations. The in vitro model of cell cultures grown on substrates of various degrees of stiffness was seen as novel and potentially useful for understanding the role of matrix stiffness in EMT. The methods used were thought to be appropriate and to reflect the state of the art. The combination of biologic and mechanical techniques for assessing interactions between airway epithelial cells and the underlying matrix and for characterizing EMT was a strength of the work.

Although the Committee had some concerns about the statistical analyses, it agreed with the investigators' overall conclusions that the results supported the hypothesis that the stiffness of the extracellular matrix drives EMT and increases cell contractility and that activation of TGF-β played a key role in EMT in the investigators' cell culture system. The results also supported the investigators' hypothesis that exposure to PM would enhance the effect of substrate stiffness on EMT and TGF-β activation.

CONCLUSIONS

Overall, the study by Barker and colleagues highlighted the potential importance of cell–matrix interactions when evaluating the effects of environmental triggers and provides a basis for future research. Considerable work will be needed to confirm these initial observations, understand the mechanisms, and determine whether they are relevant to in vivo processes and the development of fibrosis.

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