IJAE
Vol. 116, n. 1 (Supplement): 189, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

The interaction of asbestos fibres with human mesothelial cells: a combined investigation exploiting microscopic and nanoscopic techniques

Elisa Trevisan[1], Laura Andolfi[2], Barbara Troian[1], Stefano Prato[1], Claudia Casarsa[3], Francesca Vita[3], Violetta Borelli[3], Giuliano Zabucchi[3], Marina Zweyer[2]

[1] A.P.E. Research Srl, AREA Science Park, Basovizza, Trieste,
[2] Dipartimento Universitario Clinico di Scienze Mediche, Chirurgiche e della Salute, Università di Trieste,
[3] Dipartimento di Scienze della Vita, Università di Trieste.

Introduction. The exposure to asbestos fibres is associated with the development of severe diseases such as lung cancer and pleural mesothelioma. The interaction mechanism of these fibres with the mesothelial cells is still debated.(1) This work aims at obtaining information about the interaction of crocidolite fibres with mesothelial cells, for a better understanding of the processes that trigger cell transformation. For this reason we combine optical microscopy and SEM, with nanoscopic techniques as near-field optical (SNOM) and atomic force microscopy (AFM). These two latter techniques, thanks to their high sensitivity and non-invasiveness, are suitable for investigating phenomena occurring at the cell membrane with nanometric resolution.(2) In addition, SNOM provides simultaneous topography and optical image with a resolution beyond the light diffraction limit. This allows a direct coupling of the morphological features with the optical properties of the sample.

Materials and Methods. Mesothelial cell line (MET5A from ATCC) are grown in RPMI with FCS 10%, 2 mM glutamine. Cells are exposed to 5μg/cm2 crocidolite for 3, 6 or 12 h. For optical microscopy cells are stained with Diff-Quick. The samples after fixation with PFA 4% are prepared for SEM, SNOM and AFM observations that are carried out by using a Leica Stereoscan 430i, a A-100 AFM and TriA-SNOM microscope (A.P.E.Research, Trieste, Italy).

Results and Discussion. By analysing the optical data we estimate that fibres are associated with 75% of mesothelial cells. SEM images confirm these results and allow distinguishing that some fibres are on cell surface, while others appears to be clearly inside the cells, in some cases even deforming the cell morphology. A deeper investigation is achieved by SNOM and AFM. By comparing the SNOM topography with the simultaneous transmission and reflection images, we can define the position of the fibres respect to the cell membrane, owing to difference in optical properties between the crocidolite and the cell material. In addition, high-resolution AFM images highlight the entrance site of the nanometre-size fibres at cell membrane. In conclusion the combination of our findings provides an accurate description about the interaction of mesothelial cells with crocidolite fibres having different size. Importantly, SNOM optical images can disclose details about such interaction not observed up to now.

1. Arch. Biochem. Biophys. 2010, 502: 1.
2. J. Cell Sci. 2001, 114: 4153.

Keywords: mesothelial cells, crocidolite, topography, optical images, nanometre-resolution