Lamellar Inclusions within Hyperplastic Endoplasmic Reticulum in Benign Mesothelial Cells

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Keywords

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Abstract

Introduction: In effusion cytology, mesothelial cells can occasionally present with striking intracytoplasmic accumulation of rod- and crystal-like cytoplasmic lamellar inclusions (LIs). Their nature and function are poorly understood, and their diagnostic relevance is unknown. **Objective:** The aim of this study was to explore the nature of LIs in mesothelial cells and determine their prevalence and diagnostic utility in routine practice. **Material and Method:** We reviewed a consecutive series of cytological specimens of reactive ($n = 102$) and malignant effusions ($n = 90$), respectively. Malignant effusions included malignant mesotheliomas ($n = 63$) and carcinomas ($n = 27$). LIs of one effusion were analyzed by electron microscopy (EM). **Results:** LIs were found exclusively in benign mesothelial cells in 14% (14/102) of reactive and in 4% (1/27) of malignant effusions with carcinomatosis. They were absent in effusions of malignant mesothelioma. EM revealed mainly straight lamellar, less tubular, structures in cis-

ternae of the hyperplastic rough endoplasmic reticulum (rER). **Conclusion:** Cytoplasmic LIs located within hyperplastic rER can be found in up to 14% of effusions restricted to benign mesothelial cells. They can be used as an indirect morphological clue favoring the diagnosis of benign effusion and helping the cytologist to differentiate between reactive and malignant mesothelial cells in daily practice.

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Introduction

The mesothelium consists of a monolayer of specialized pavement-like cells that line the entire surface of the 3 serosal cavities (pleural, pericardial, and peritoneal), providing a nonadhesive and protective surface [1]. Besides this protecting role, they are also implicated in the transport of soluble substances and cells across serosal cavities, in antigen presentation and in tissue repair [1].

We have observed a number of benign effusions containing mesothelial cells with rod-shaped, crystal-like la-

Fig. 1. a–d Conventional cytology (Pap stain and MGG stain) highlighting mesothelial cells filled up with LIs (yellow arrows) ($\times 630$, Pap (**a**); $\times 630$, Pap (**b**); $\times 630$, Pap (**c**); $\times 630$, MGG (**d**)). Pap, Papanicolaou; MGG, May-Grünwald-Giemsa; LIs, lamellar inclusions.

mellar inclusions (LIs). Such LIs have previously been described by Zaharopoulos et al. [2]. However, their nature and function in mesothelial cells remain poorly understood, and their prevalence and clinical implications are unknown. To determine their prevalence in benign and malignant effusions, we reviewed pleural and peritoneal effusion cytology findings of reactive and malignant effusions for the presence of LIs. Electron microscopy (EM) was applied to elucidate the nature of LIs.

Material and Method

Review of Cases

We reviewed a consecutive series of 102 Papanicolaou (Pap)-stained benign pleural and peritoneal effusion specimens, diagnosed with reactive mesothelial cells and 27 cases with pleural or peritoneal carcinomatosis, diagnosed between 2017 and 2018. Further, we reviewed 63 Pap-stained cytological pleural specimens diagnosed with malignant mesothelioma between 2010 and 2018. All the samples reviewed were Pap-stained smear. For each case, one Pap-stained smear was screened for at least 1 min using a $\times 20$ objective. Suspected LIs were confirmed at a magnification of $\times 63$.

Transmission EM

One index case with abundant LIs was further analyzed with transmission EM. For EM, the cells were fixed in 2% buffered glutaraldehyde at room temperature for 2.5 h, then postfixed in 1% OsO_4 for 1 h and embedded according to standard procedures in Spurr's epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a Zeiss 10C transmission electron microscope.

Immunocytochemistry

All the cells with LIs were characterized by immunocytochemistry using antibodies against calretinin (Clone Cal 6) and/or CD68 (Clone PG-M1) on previously Pap-stained specimens using the Leica Bond 3 automated immunostainer.

Results

Light Microscopic Definition of LIs in Mesothelial Cells

LIs are defined as rod-shaped, crystal-like, non-birefringent structures distributed in a perinuclear distribution or occupying and expanding most of the cytoplasm (shown in Fig. 1a–d). LIs stained weakly reddish or purple

Fig. 2. **a, b** Conventional cytology (Pap stain) with aggregate of mesothelial cells filled up with LIs ($\times 630$, Pap). **c, d** Immunocytochemical stain showing diffuse cytoplasmic and nuclear positivity for calretinin and negativity for CD68 of the above depicted cells ($\times 630$, calretinin (**c**); $\times 630$, CD68 (**d**)). Pap, Papanicolaou; LIs, lamellar inclusions.

in the Pap-stained smears that had been fixed with Delaunay solution (1,000 mL each of acetone and absolute alcohol and 20 drops of 1 mol/L of trichloroacetic acid) (shown in Fig. 1a–c). Increasing the contrast by adjusting the aperture of the condenser enhances the typical rod-shaped or crystal-like structure of LIs. Cases with prominent LIs were also detectable in air-dried May-Giemsa-Grünwald specimens, where they appear as bluish perinuclear densities (shown in Fig. 1d). The mesothelial nature of the cells was confirmed by immunochemistry showing a diffuse positivity for calretinin and negativity for CD68 (shown in Fig. 2a–d).

Electron Microscopy

EM revealed that LIs were composed of mainly straight rarely curved bi- or multilayered lamellae or tubules in the cisternae of the hyperplastic rough endoplasmic reticulum (rER), as pictured and described by Ghadially [3]. These structures belong to the microtubule group of ordered arrays in the rER (shown in Fig. 3a–c). This interpretation was confirmed by several experts in EM analysis.

Prevalence of LIs

Benign mesothelial cells containing LIs were found in 13.7% (14/102) of all benign reactive effusions. LIs were more common in pleural effusions (18%; 13/72) than in

peritoneal effusions (3%; 1/30). Benign mesothelial cells with LIs were found in only 4% (1/27) of cases with pleural carcinomatosis. In addition, no LIs were present in 63 effusions from malignant mesothelioma ($n = 63$). The number of LI-positive mesothelial cells in LI-positive effusions varied from very few to numerous but was always less than an estimated 30% of all mesothelial cells. LIs were restricted to mesothelial cells and were not found in macrophages.

Discussion

In this study, we describe cytoplasmic lamellar rod-shaped, crystal-like inclusions which are present only in benign mesothelial cells. They are found in up to 18% of benign pleural and, less often, in peritoneal effusions. EM analysis shows that these LIs represent structures of hyperplastic rER. These structures belong to the microtubule group of ordered arrays in the rER.

LIs have previously been described by Zaharopoulos et al. [2] in 3 patients with benign effusions. They postulated that LIs were membranes of erythrocytes engulfed/phagocytosed by mesothelial cells or macrophages [2]. In our study, LIs were only seen in mesothelial cells but not in macrophages, and no signs of erythrophagocytosis by mesothelial cells were found. Although it has been dem-

Fig. 3. **a** Overview showing mesothelial cells with the cell in the upper part containing abundant LIs, which are distributed in a perinuclear fashion. Cells in the lower part without LIs (TEM, $\times 4,400$). **b, c** Higher magnification of the LIs of the cells shown before. The LIs consist of parallel arranged lamellae or tubules in the cisternae of the rER. Ribosomes connected to the rER as well as free ribosomes can also be appreciated. $\times 11,000$, TEM (**b**); $\times 71,000$, TEM (**c**). TEM, transmission electron microscopy; rER, rough endoplasmic reticulum; L, lysosome; LIs, lamellar inclusions; N, nucleus; M, mitochondria; R, ribosomes; FR, free ribosomes; ER, endoplasmic reticulum.

onstrated that human peritoneal mesothelial cells are capable of engulfing dying ovarian and colorectal cancer cells and, as such, mesothelial cells can, therefore, exhibit phagocytic capability, our EM studies clearly rule out the above hypothesis [4]. Normal mesothelial cells contain glycogen, microtubules and microfilaments, few mitochondria, a poorly developed Golgi apparatus, and very little ER [1]. Following injury or any stimulation of the serosal surfaces, an activation of the cells may occur with abundant cell organelles such as mitochondria, well-developed ER, and prominent Golgi apparatus [5, 6]. The cause of hyperplasia of rER in mesothelial cells remains unclear. ER is the largest organelle of eukaryotic cells and has numerous functions. For example, it plays a vital role in the synthesis of proteins, lipids, and glycogen; mediates the transport of proteins to other organelles and the cell membrane; and also serves as a Ca^{2+} storage site [7]. These functions suggest that rER hyperplasia might result from an increased metabolism and protein or lipid synthesis of the mesothelial cells in the context of reactive changes. Apparently, this reaction seems to be a physiological process of benign cells, as we

did not observe it in mesothelioma cells. Clearly, further studies are needed to identify the underlying causes of hyperplasia of the rER in mesothelial cells. The cause of the difference in frequency noted between reactive pleural and peritoneal mesothelial cells in respect of LIs also remains unclear.

Differentiating reactive from malignant mesothelial cells can be challenging as malignant cells can sometimes be morphologically indistinguishable from benign, reactive mesothelial proliferations, or even from macrophages. In this study, we showed that although relatively rare, the presence of LIs in mesothelial cells could be used as an indirect morphological clue favoring benign changes and can, thus, help the cytologist in differentiating between reactive and malignant mesothelial or epithelial cells. However, it should be emphasized that the reason why this phenomenon occurs with varying frequency in tumor-free effusions is not clear. In summary, we have shown that LIs in mesothelial cells are part of hyperplastic rER and that their presence in mesothelial cells can be used as a simple, adjunct morphological sign of benignity in daily practice.

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Statement of Ethics

This study was covered by the local institutional approval EK 253/08 on human research (“Ethikkommission beider Basel”). The subjects gave their written informed consent.

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Conflict of Interest Statement

The authors have no conflict of interest to declare.

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Author Contributions

L.B. conceived and designed the project. J.D., S.H., T.M., H.H., M.J.M., and L.B. analyzed the data. S.H. and L.B. wrote the draft manuscript. T.V., M.J.M., and S.S. worked on the manuscript. All authors agreed to the content of the manuscript.