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Paracrystalline inclusions in metaplastic ciliated cells of the human gastric mucosa

An ultrastructural study

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Summary. Unusual electron-dense paracrystalline inclusions were found in metaplastic ciliated cells in the stomachs of three Japanese male patients with gastric carcinoma. These patients had not been given antitumour drugs before surgery and ethrane (enflurane) was used as the anaesthetic. Ciliated cells in the gastric mucosa are found not infrequently in the pyloric glands in association with intestinal metaplasia in elderly Japanese patients. Paracrystalline inclusions were found only in the ciliated cells and never in any other types of gastric mucosal cell. These inclusions were located in the apical portion of the ciliated cells in intimate association with the basal bodies. They consisted of twisted strings about 27 nm wide with a regularly repeated spacing of about 30 nm. On highly magnified electron micrographs, granules about 4 nm in diameter were detected. These paracrystalline inclusions have never been reported previously, although their location in ciliated cells and their morphological characteristics suggest an intimate relationship with the ciliogenesis of metaplastic ciliated cells in the human stomach.

Key words: Paracrystalline inclusion – Microtubule – Ciliogenesis – Gastric ciliated cell – Ultrastructure

Introduction

Cilated cells are present in the human stomach, mainly in the pyloric glands in association with intestinal metaplasia, and are thought to represent a type of metaplasia with a relatively high incidence among Japanese patients (Torikata et al. 1986; Rubio and Kato 1986; Kawakita 1987). Electron microscopy has revealed that the fine structure of these gastric cilia is almost the same as that of respiratory cilia (Torikata et al. 1989).

Unusual electron-dense inclusions, some showing a paracrystalline appearance, were found in the apical portion of gastric ciliated cells in three Japanese patients. These structures sometimes had a close relationship with the basal bodies.

Crystalline inclusions in various types of human cells have been studied by electron microscopy, such as Reinke's crystals in Ledig's cells (Fawcett and Burgos 1966; Nagano and Ohshima 1971), renin crystals in juxtaglomerular cells (Biava and West 1966), and crystalloids of Charcot-Böttcher in Sertoli cells (Nagano 1966). Tumour cells of alveolar soft part sarcoma contain numerous crystalline inclusions in the cytoplasm (Shipkey et al. 1964). Additionally, numerous unidentified paracrystalline inclusions have been found in neoplastic cells (Ghadially 1988). Despite the numerous studies the significance of these crystalline inclusions remains obscure.

Tubulin crystals have been reported in vincristine-treated (Bensch and Malawista 1969; Tyson and Bulger 1973; Dustin et al. 1980) and colchicine-treated cells (Schechter et al. 1976; Bennett and Smith 1979) and in the normal spermatids of some stick-insects (Afzelius 1988). Also, microtubule-macrotubule transformation has been induced by volatile anesthetics (Hinkley and Samson 1972; Hinkley 1976). However, the unusual paracrystalline inclusions found in the gastric ciliated cells in the current study were quite different. No crystalline inclusions have been reported in the respiratory ciliated cells and this typed of inclusion has never been reported previously, even in non-ciliated cells.

In this paper, we describe the fine structure of these paracrystalline inclusions of gastric metaplas-

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tic ciliated cells and discuss the posible nature of their components and mechanism of formation.

Materials and methods

Gastric mucosae used in this study were obtained from surgical specimens resected from patients with gastric cancer. No patients had been administered any antitumor drugs before their operations and ethrane (enflurane) was used as the anesthetic in all three cases. Small pieces of the pyloric mucosa, one of which was formalin-fixed, were fixed with 1% tannic acid containing 1% glutaraldehyde (Mizuhira and Futaesaku 1972) and post-fixed with 1% osmium tetroxide, dehydrated in a graded ethanol series and acetone and embedded in Spurr's resin (Spurr 1969). Ultrathin sections were cut and double-stained with uranyl acetate and lead citrate, and examined with a JEOL 100C electron microscope at 100 kV.

None of the three patients had a history of preoperative administration of antitumour drugs and ethrane (enflurane) was used as the anesthetic at surgery.

Results

In the pyloric mucosa, ciliated cells were found in the basal part of the glands and never in the surface or foveolar epithelium. They were always found in association with so-called intestinal metaplasia. However in the basal part, ciliated cells were sometimes located among normal-looking mucous cells, singly or in small clusters, and mitotic cells were found in the neck of the gland. Occasionally many ciliated cells were lined up along the dilatated glands and contained mucus-negative vacuoles in the perinuclear region (Fig. 1).

On electron microscopy, cilia were found on the cell surface and in the concave channels, mixed with fairly long microvilli. Gastric cilia consisted of three major components; the ciliary shaft, the neck and the basal body, with a fine structure similar to that of respiratory cilia. However, most of the dynein arms were inconspicuous, even after tannic acid fixation. An abnormal arrangement and/or number of microtubules in an axoneme was also observed not infrequently among these gastric cilia, with a few abnormalities of the basal bodies (Fig. 2).

Unusual electron-dense paracrystalline inclusions were found in the present three male patients (63, 68 and 73 years old) with gastric carcinoma. These paracrystalline inclusions were found in the apical portion of the ciliated cells in close association with the basal bodies and were sometimes connected with the top of the basal foot. However, they were never found in other types of cell in the gastric mucosa (Fig. 3). The inclusions consisted of electron-dense material without a limiting unit membrane and sometimes showed a paracrystalline structure. In some sections, they presented a ladder-like structure in which electron-dense and -lucent bands were regularly arranged with a repeating lattice of about 30 nm. However, in other sections, long twisted strings about 27 nm wide were apparent, showing an electron-dense periodicity of about 30 nm (Fig. 4). On high-magnification electron micrographs, small clustered granules about 4 nm in diameter were seen, showing a partially regular arrangement (Fig. 5).

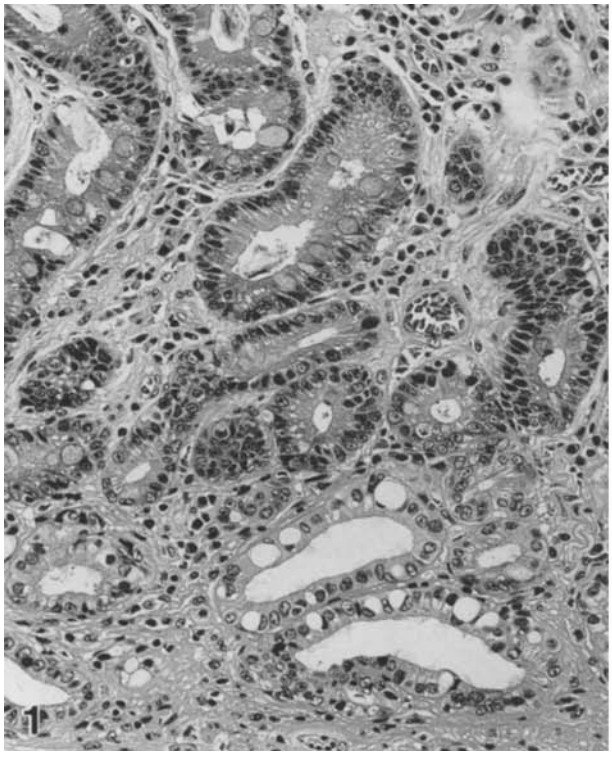
Discussion

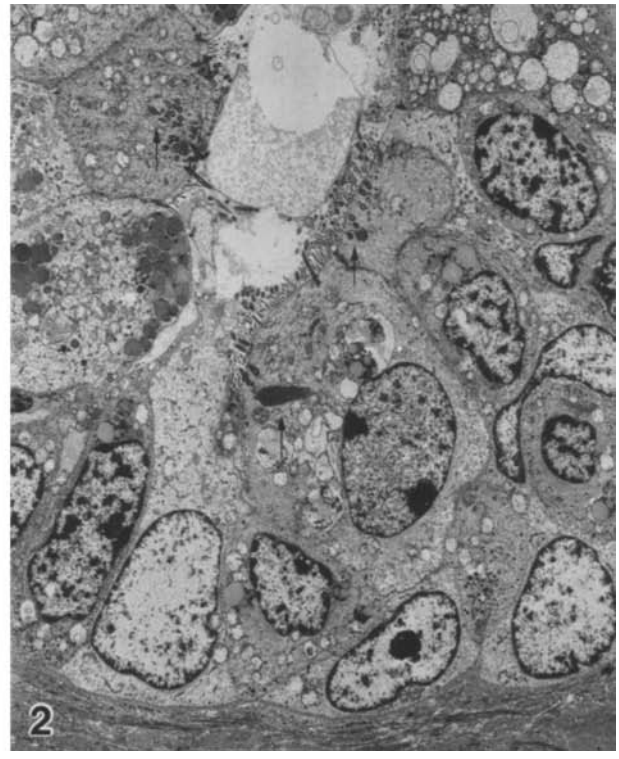
Ciliated cells in the human gastric mucosa were always found in association with intestinal metaplasia, mainly in the pyloric mucosa. They were present in the basal part of the glands but never in the surface or foveolar epithelium. This made it difficult to observe the cells directly by light microscopy for determination of ciliary motility in the stomach. The occurrence of ciliated cells in the human stomach is thought to be a form of metaplasia of epithelium, but the functional and pathological significance of metaplastic ciliated cells has not been clarified, and the reason why they are so common in Japanese patients still remains unsolved.

By electron microscopy, the fine structure of the gastric cilia was almost the same as that of respiratory motile cilia, but most of the dynein arms of the gastric cilia were inconspicuous even after tannic acid fixation. Additionally, various type of abnormal cilia have been found among gastric cilia, but compound cilia, a common type of acquired abnormality among respiratory cilia, were never found in the stomach (Torikata et al. 1989).

Crystalline and paracrystalline inclusions have been found in normal and neoplastic human cells (Ghadially 1988). Reinke's crystals in the Ledig's cell of the human testis have been studied by electron microscopy (Fawcett and Burgos 1960; Nagano and Ohtsuki 1971) and have also been found in the ovarian hilar cells. Reinke's crystals are believed to be a storage form of protein but the nature of the protein is unknown. Crystals in the juxtaglomerular cells of the human kidney and in juxtaglomerular cell tumours have been revealed in an intimate relationship to renin (Biava and West 1966; Phillips and Mukherjee 1972) and crystals in alveolar soft part sarcoma relate to actin (Mukai et al. 1984, 1986).

Microtubular or tubulin crystal formation has been induced by colchicine (Schechter et al. 1976; Bennett and Smith 1979) and vinca alkaloids





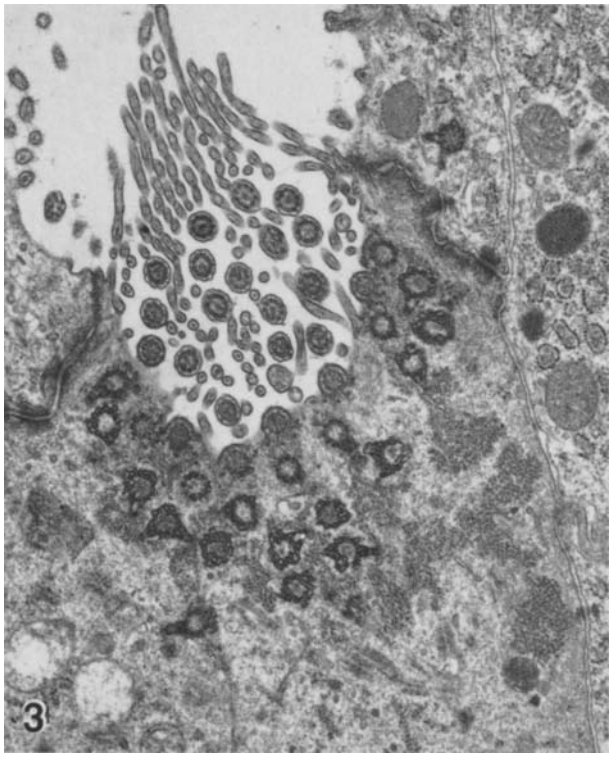
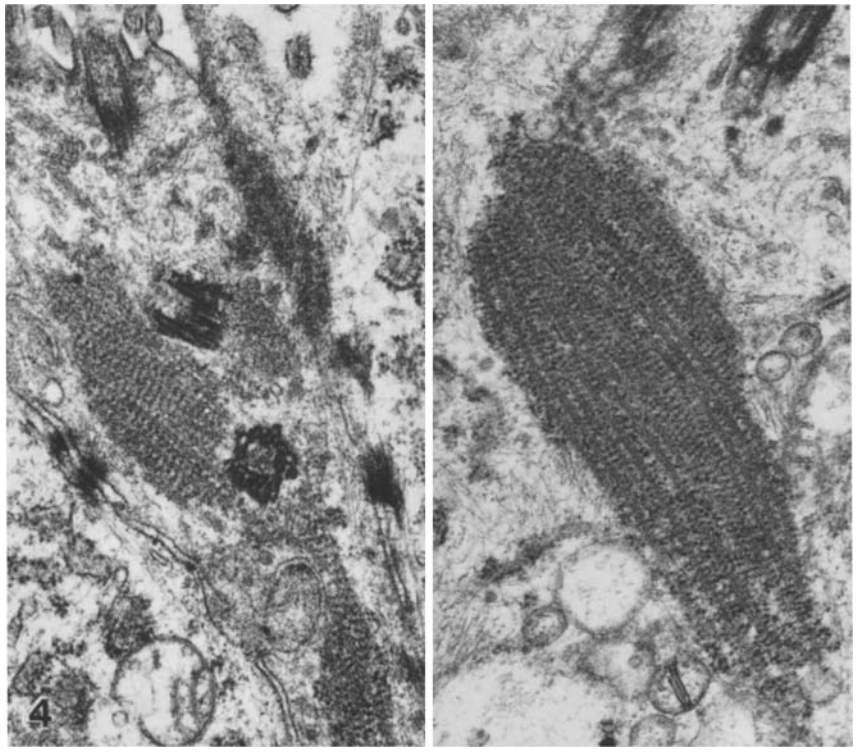


Fig. 1. Ciliated cells in the metaplastic pyloric mucosa of the human stomach. Ciliated cells are noted in the basal part of the metaplastic pyloric gland and sometimes contain large mucus-negative vacuoles. Numerous cilia are present on the apical surface. H–E ×200

Fig. 2. Ciliated cells in the human pyloric mucosa. Ciliated cells present among pyloric mucus cells, mucus neck cells and neuroendocrine cells. The ciliated cells contain prominent autophagosomes in the supranuclear region and abundant cytofilaments. Cilia and microvilli are present on the cell surface and unusual electron-dense inclusions are noted in the apical portion of the ciliated cells (*arrow*). ×2400

Fig. 3. Unusual electron-dense inclusions of the ciliated cells in the human pyloric mucosa. Numerous cilia mixed with fairly long microvilli and basal bodies are noted in the apical portion of the cells. Electron-dense inclusions are seen around the basal bodies and some of them seem to have an intimate relationship with the top of the basal foot. They consist of parallel filamentous components and electron-dense materials. ×15000

(Bensch and Malawista 1969; Tyson and Bulger 1973; Dustin et al. 1980). They have recently been found in the normal spermatids of some stick-insects (Afzelius 1988). Bensch and Malawista (1969) documented microtubule crystals in the centromeres of cells in close association with the centriole; a microtubule organizing center. These crystals also contained ribosomes. Volatile anesthetics,



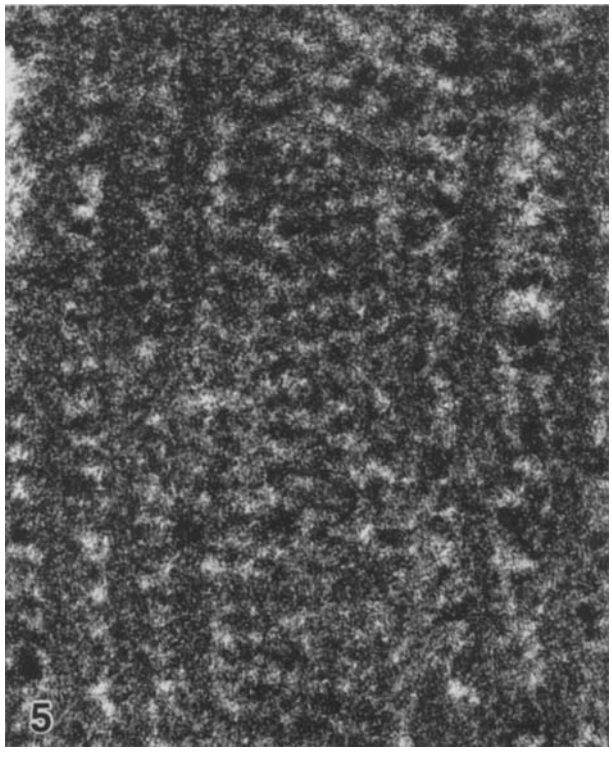


Fig. 4. Paracrystalline inclusions of a metaplastic ciliated cell of the human pyloric mucosa. Unusual electron-dense inclusions are evident around the basal bodies and show regularly arranged, black and white bands with a repeat lattice of about 30 nm and twisted strings about 27 nm wide. × 30000

Fig. 5. Paracrystalline inclusions of a metaplastic ciliated cell in the human pyloric mucosa. Small granules about 4 nm in diameter are clustered on the strings. In some areas, they appear to have a regular arrangement. × 150000

including halothane and ethrane induce microtubule-macrotubule transformation (Hinkley and Samson 1972; Hinkley 1976) and zinc forms flat sheets of tubulin (Larsson et al. 1976; Tamm et al. 1979) in vitro. Polymorphic assemblages of tubulin have been reviewed by both Burton (1981) and Dustin (1984), but most have been experimentally induced.

However, there have been no reports on unusual electron-dense paracrystalline inclusions like those found in this study. As described previously, these inclusions were found in the apical portion of the metaplastic ciliated cells and sometimes had an intimate relationship with the basal bodies, being connected directly to the top of the basal foot. In normal respiratory cilia, a few disorganized microtubules connected with the top of the basal foot are observed not infrequently, but their physiological function is unclear. Because of their location in ciliated cells and their close relationship with basal bodies, these paracrystalline inclusions seem to be intimately related to both.

In normal ciliogenesis, fibrogranular elements of unknown provenance, known as filosomes, are noted in the supranuclear region and may form deuterosomes or the precentriole-organizer (Youson 1982). However, the ultrastructure of the present paracrystalline inclusions was quite different from that of filosomes.

From our electron microscopic findings, these inclusions seem to have an intimate relationship with tubulin molecules or component proteins of the basal bodies, and are possibly produced due to the effects of volatile anesthetics. Additionally, if this type of inclusion is induced experimentally, further fine structural and histochemical studies might reveal its nature.

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