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ULTRASTRUCTURAL EVIDENCE OF EOSINOPHIL CLUSTERING AND ETOSIS IN ASSOCIATION WITH DAMAGE TO SINGLE TUMOUR CELLS IN A CASE OF POORLY COHESIVE NOS GASTRIC CARCINOMA

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ABSTRACT

A case of poorly cohesive NOS gastric carcinoma, characterised by high-grade tumour-associated tissue eosinophilia (TATE), is studied by transmission electron microscopy. Eosinophil clustering around single tumour cells constituted a recurrent ultrastructural hallmark. Some eosinophils were in intimate contact with tumour cells and exhibited extracellular trap cell death (ETosis): a non-apoptotic cell death process, recently described in non-neoplastic, eosinophil-associated diseases. Discharge of chromatin material and specific granules, due to eosinophil ETosis, was polarised towards single tumour cells that showed various degrees of cytopathogenic changes. Our data suggest that eosinophil ETosis may exert an antitumoural activity in gastric cancer.

KEYWORDS

Gastric carcinoma; eosinophil ETosis; tumour cell injury; ultrastructure

LEARNING POINTS

- A recent meta-analysis reported that TATE is a histopathological marker of favourable prognosis, particularly in patients with gastrointestinal cancer.
- Experimental studies have shown that eosinophils may exert antitumour activity through discharge of their highly cytotoxic granular proteins.
- Our ultrastructural findings add novel mechanism insights for eosinophil antitumoural activity, providing morphologic evidence of eosinophil ETosis in association with single tumour cell injury.

INTRODUCTION

Human eosinophils are innate immune cells that mainly infiltrate tissues characterised by cell proliferation and death, as in normal injury repair, helminth parasite infections, allergic diseases, non-atopic diseases and cancer[1]. Ultrastructural studies have shown distinct mechanisms

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of eosinophil degranulation including classical exocytosis, compound exocytosis, piecemeal degranulation, cytolysis and extracellular trap cell death (ETosis)[1]. Eosinophil ETosis is a non-apoptotic type of programmed cell death culminating in extracellular discharge of specific granules and nuclear chromatin in the form of extracellular traps[1]. Tumour-associated tissue eosinophilia (TATE) is a term coined by pathologists to describe increased numbers of eosinophils in some tumours in the absence of necrosis or ulceration[1]. Recently, a meta-analysis revealed that TATE is a favourable prognostic marker for human solid tumours[2].

In our previous ultrastructural studies on human gastric carcinomas with TATE we reported eosinophil degranulation patterns, such as piecemeal degranulation and cytolysis, as well as classical and compound exocytosis[3]. In the present work, we add a new case of gastric carcinoma where eosinophil ETosis constituted the principal pattern of degranulation. The possible involvement of ETosis in single tumour cell death is discussed according to current literature data.

CASE REPORT

A 71-year-old woman was admitted to our hospital for nausea, weight loss and anaemia. Endoscopic examination of the upper gastrointestinal tract revealed a plaque-like lesion with flattening of the rugal folds in the gastric antrum, in the absence of ulceration. Histological diagnosis of biopsy specimens was poorly cohesive gastric carcinoma. Computed tomography did not reveal distant metastases. The patient underwent a total gastrectomy with gastrojejunostomy and lymph node dissection in January 2022. The post-operative course was uneventful. The patient received cycles of chemotherapy and at the 15-month follow-up, the patient was alive without evidence of recurrence.

MATERIALS AND METHODS

Gastric tumour was processed for both light and electron microscopic studies. Semithin sections were stained with Toluidine blue for selection of fields. For electron microscopy, ultra-thin sections were double stained with uranyl acetate and lead citrate; they were then examined under a JEOL 1200 electron microscope (JEOL, Tokyo, Japan) at 70 kV.

RESULTS

Gross findings

Grossly, a Borrmann type 4 gastric cancer was confined to the gastric antrum. The tumour lesion was not associated with ulceration and showed a diffuse wall thickening.

Histologic findings

Gastric cancer consisted of single or small clusters of tumour cells that showed a vaguely histiocytic appearance. Tumour cells were embedded in a desmoplastic collagenous stroma, and invaded the mucosal, submucosal, muscular and subserosal layers of the stomach. They exhibited a large nucleus and a scant to moderate amount of eosinophilic cytoplasm, devoid of mucin granules. TATE was also present *(Fig. 1)*. None of the 16 regional lymph nodes exhibited metastasis and no distant metastasis was detected. The pathological diagnosis was poorly cohesive carcinoma non-signet ring cell type (NOS) according to WHO classification 2019[4] (pT3, N0, M0, stage IIA).

Ultrastructural findings

Eosinophils formed heterotypic aggregations with single tumour cells. Early phases of eosinophil ETosis including chromatin decondensation, dilation of nuclear envelope and discharge of specific granules were revealed by electron microscope at the site of contact with undamaged single tumour cells *(Fig. 2)*. Figure 3 shows polarisation of both specific granules and decondensed chromatin in the eosinophil cytoplasm adjacent to a single tumour cell. A tumour cell in contact with eosinophils exhibited early cytopathic changes such as cytoplasmic vacuoles and mitochondrial swelling *(Fig. 3)*. In a more advanced phase of eosinophil ETosis, there was discharge of specific granules and decondensed chromatin corresponding to

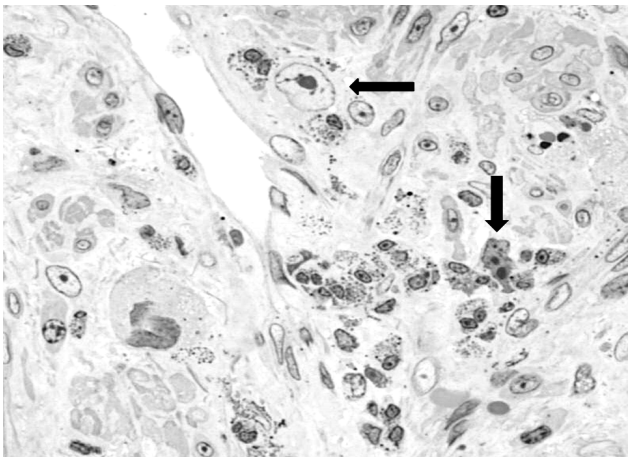

Figure 1. Semithin section showing histiocyte-like discohesive tumour cells. Note the eosinophils surrounding single tumour cells (arrows)

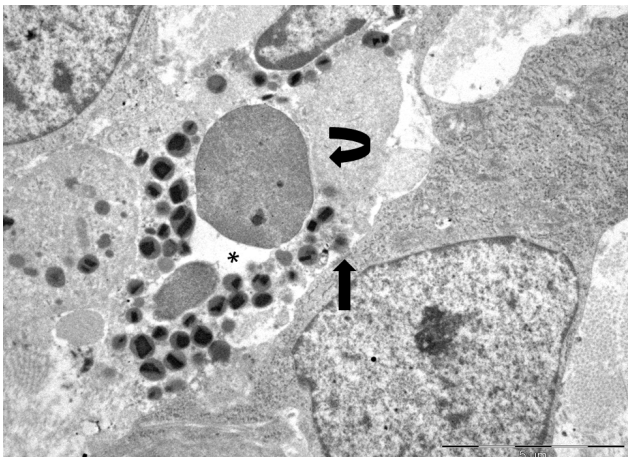

Figure 2. Eosinophil in contact with a single undamaged tumour cell shows early ultrastructural signs of ETosis characterised by decondensed nucleus (curved arrow), dilation of nuclear envelope (asterisk) and extracellular release of free specific granules at the region of contact with the tumour cell (arrow)

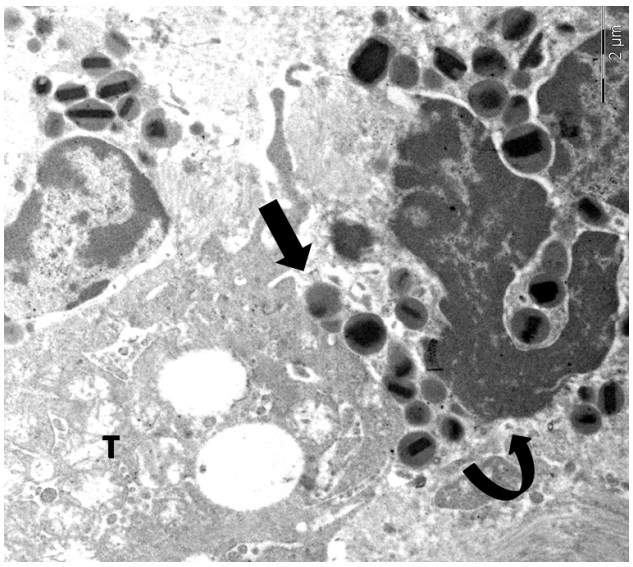

Figure 3. Heterotypic eosinophil-single tumour cell aggregation. Note the polarisation of specific granules (arrow) and decondensed nucleus (curved arrow) in the eosinophil cytoplasm in proximity to the contact site with a single tumour cell (T) that shows early cytopathic changes such as cytoplasmic vacuoles and mitochondrial swelling

the site of contact with single tumour cells. This showed severe cytopathic changes including perinuclear and rough endoplasmic cisternae dilation, extensive cytoplasmic vacuolisation, focal disruption of cell membrane and nuclear chromatin condensation, but without margination of chromatin *(Fig. 4A)*. Pyramidal or hexagonal Charcot-Leyden crystals were focally observed in the tumour stroma *(Fig. 4B)*.

DISCUSSION

Our ultrastructural data show heterotypic cell aggregations where ETotic eosinophils were in intimate contact with damaged single tumour cells, showing progressive cytopathic changes. In particular, polarisation of specific granules and decondensed chromatin is documented at the region of intimate contact with tumour cells, where the plasma membranes of the respective cells are focally disrupted. Our ultrastructural findings of Charcot-Leyden crystals are in accordance with previous studies showing their association with eosinophil ETosis[1].

Eosinophils may exert antitumour activity in vitro through the release of cytotoxic granular proteins[1]. Experimental studies show that eosinophil cationic proteins aggregate on the cell membrane, forming pores that determine osmotic cell lysis[5]. Accordingly, single tumour cells in contact with ETotic eosinophils show ultrastructural signs of paraptosis, such as mitochondrial swelling, dilation of nuclear envelope, cytoplasmic vacuoles and nuclear chromatin condensation[6]. Paraptosis is a non-apoptotic mechanism of programmed cell death, correlated to osmotic cell lysis[6]. Taken together, these literature data suggest that ETotic eosinophils are responsible for the paraptosis-like cell death mechanism in single tumour cells, due to osmotic cell lysis.

In conclusion, we present eosinophil ETosis for the first time in a human malignant tumour. Eosinophil ETosis is considered

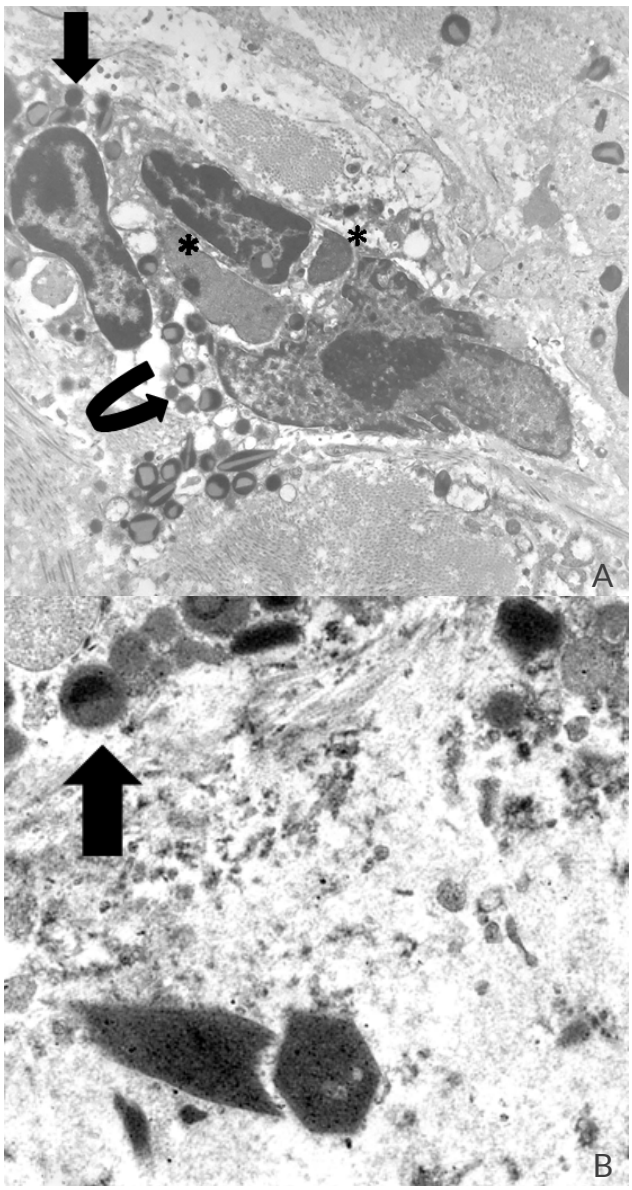

Figure 4. (A) An eosinophil undergoing ETosis is in intimate contact with a tumour cell, showing advanced cytopathic changes including extensive cytoplasmic vacuolisation, focal loss of plasma membrane and nuclear chromatin condensation. Polar degranulation of eosinophil is suggested by the presence of decondensed nucleus (asterisks) and free extracellular granules (curved arrow) in the area of intimate contact with the tumour cell. Note the adjacent intact eosinophil (arrow) showing a bilobed nucleus where euchromatin and heterochromatin are well distinguishable

(B) Pyramidal or hexagonal Charcot-Leyden crystals are noted near free extracellular granules (arrow)

to be an innate immune function as the first line of defence against non-phagocytosable organisms such as parasites and fungi[1]. Our ultrastructural findings confirm and extend these observations that include ETosis as an antitumoural mechanism also for non-phagocytosable single carcinoma cells.

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