DOI: 10.25205/978-5-4437-1843-9-291

STAGES OF LYSOSOMAL ENTOSIS IN CANCER TISSUE LUNG AND COLORECTAL CANCER

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Abstract

Entosis is a process in which viable cells are internalized into neighboring cells of the same type, involving adhesion molecules, while forming cell-in-cell (CIC) structures. It is noted that this behavior is typical for some types of tumors. There are no studies in the literature demonstrating the stages of entosis on the clinical material of cancer patients.

Introduction

In 2007, Overholtzer M. et al. published an article that presented a new process of non-apoptotic cell death in human tumors caused by loss of adhesion to the matrix [1]. Entosis is a non-apoptotic type of cell death, where the internal cell itself actively penetrates the host cell. Entosis occurs between homotypic cells with the participation of adhesion molecules, forming cell-in-cell structures (CIC). Chemotherapy is believed to be able to induce entosis between tumor cells. We assume that under the influence of chemotherapy, due to the entosis mechanism, a fusion of the genetic material of the internalized and engulfing cells can occur, which provokes the occurrence of aneuploidy, in particular, the amplification of stem cell genes [2].

Also in 2017, Garanina A. S. et al. identified 5 stages of lysosomal entosis in situ [3]. There are no studies in the literature demonstrating these stages directly on clinical material from cancer patients.

Material and methods

This study included 40 patients with colorectal cancer (CRC) T1-4N0-2M0-1 and 30 patients with lung cancer (LC) T1-3N0-3M0-1 with a morphologically verified diagnosis. The patient study material was fixed, after which paraffin blocks were made for histological sections. Then standard hematoxylin and eosin staining was performed. For the histological determination of entosis, the Mackay criteria were used [4], which are recognized and used by many laboratories.

The frequency of entosis was compared with the main clinical and morphological parameters of patients (stage, tumor size, degree of differentiation, Grade). Statistical data processing was performed using the Statistica 8 software package (StatSoft Inc., USA). After that, one sample from each localization was selected for visualization of entosis on a 3D HISTECH PANNORAMIC MIDI histoscanner. Statistical data processing was performed using the Statistica 8 software package (StatSoft Inc., USA).

Results

For each slide, twenty randomly selected fields of the histological preparation were taken, in which the search and counting of entotic structures was carried out (fig. 1). The average frequency of occurrence in patients with CRC was 0.25, and in patients with LC — 0.07.

Next, we analyzed the relationship between the frequency of entotic events with the main clinical and morphological parameters of patients: tumor size, stage, degree of differentiation, degree of malignancy. As a result of the analysis, it was revealed that the frequency of entotic events practically did not correlate with the clinical and morphological parameters. A relationship was shown between the age of patients and the number of CICs ($p = 0.007$) in CRC, as well as between lymphogenous metastasis and the number of CICs ($p = 0.03$) in LC.

The prognostic significance of CIC structures was also assessed (fig. 2). The overall survival of colorectal cancer patients and metastasis-free survival of lung cancer patients were calculated. In patients with LC who did not have entoses, metastasis-free survival was almost 80 %, in patients with entoses this indicator was 2 times lower ($p = 0.05$).

To visualise the results, we selected one sample for each localisation with a CIC frequency of more than 0.3 in the clinical material. In the process, we were able to identify 5 consecutive stages of lysosomal entosis.

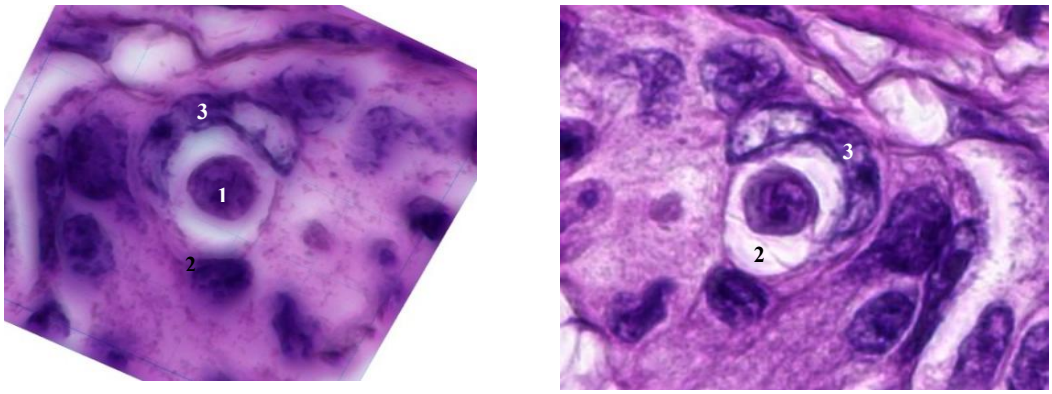

Fig. 1. Entosis images obtained with 3D HISTECH PANNORAMIC MIDI:
1 — the nucleus of the inner cell; *2* — entotic vacuole; *3* — the nucleus of the host cell

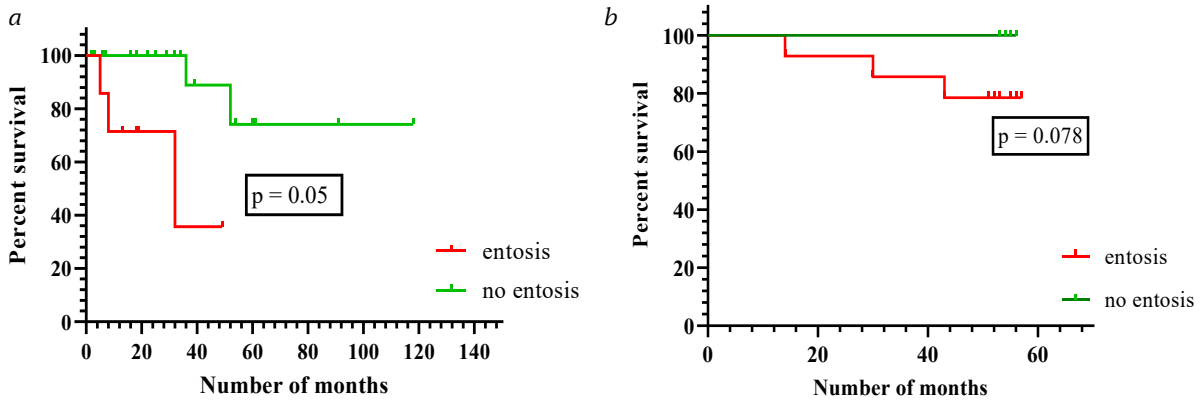

Fig. 2. Graph of metastatic-free survival of lung cancer patients (*a*)
and graph of overall survival of colorectal cancer patients (*b*)

Conclusion

In connection with the results obtained, the study of entosis will be continued; it may provide a key to understanding the mechanism of rapid development of aneuploid subpopulations of tumour cells during chemotherapy and tumour progression. We have also managed to confirm the existence of the stages of entosis in cell cultures, which have been identified in the literature.

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