

CYTOLOGICAL EFFECTS IN LYMPH NODES OF ABDOMINAL LYMPHODISSECTION ZONE AFTER INTRAOPERATIVE PHOTODYNAMIC THERAPY OF GASTROINTESTINAL CANCERS

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Abstract

Cytological studies on lymph nodes of abdominal lymphodissection zone after local intraoperative photodynamic therapy (IOPDT) of gastrointestinal cancers were carried out. As a result of the PDT, the metastatic cells are destroyed, their cytoplasmic membranes and the cytoplasm disappears, leaving behind interphase nuclei ("naked nuclei") ($p < 0,0001$). Cytological confirmation of apoptosis (the presence of apoptotic bodies) in metastatic lymph nodes after IOPDT sessions on the lymph nodes of the abdominal lymphodissection zone is also presented.

Keywords: photodynamic therapy, cytopathology, interphase nucleus, apoptosis, radachlorin, fotoditazin.

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ЦИТОЛОГИЧЕСКИЕ ЭФФЕКТЫ В ЛИМФАТИЧЕСКИХ УЗЛАХ ЗОНЫ АБДОМИНАЛЬНОЙ ЛИМФОДИССЕКЦИИ ПОСЛЕ ИНТРАОПЕРАЦИОННОЙ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ ПРИ ЗЛОКАЧЕСТВЕННЫХ НОВООБРАЗОВАНИЯХ ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА

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Резюме

Проведены цитологические исследования материала лимфатических узлов зоны абдоминальной лимфодиссекции после интраоперационной фотодинамической терапии (ИОФДТ) при злокачественных новообразованиях желудочно-кишечного тракта. Установлено, что в результате проведения ФДТ клетки метастатических опухолей разрушаются с исчезновением цитоплазматической мембраны и цитоплазмы, при этом остаются только интерфазные ядра («голые ядра») ($p < 0,0001$). Также представлено цитологическое подтверждение апоптоза (наличие апоптотических телец) в лимфатических узлах с метастазами после курсов ИОФДТ в лимфатических узлах зоны абдоминальной лимфодиссекции.

Ключевые слова: фотодинамическая терапия, цитопатология, интерфазные ядра, апоптоз, радахлорин, фотодитазин.

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Introduction

A study of lymph nodes of the zone of abdominal lymph node dissection performed after intraoperative photodynamic therapy (IOPDT) with the radahlorin or fotoditazine photosensitizer (PS) to detect apoptosis in case of malignant tumours of the gastrointestinal tract was described in the previously published article. Using DNA electrophoresis, it was proved that apoptosis after PDT is induced in lymph nodes affected by metastases and does not occur in intact lymph nodes ($p < 0.01$). This fact indicates the selective ability of PDT to cause the death of malignant cells [1].

In this study, cytologic preparations used in the previous stages to detect metastases were analyzed again under immersion. The purpose of this retrospective study was to search for cytological manifestations of apoptotic (apoptotic bodies) and necrotic (cytonecrosis, phagocytic infiltration) processes in populations of metastatic lymph node cells induced by PDT.

Materials and methods

The groups of examined patients and their diagnoses, the procedure for PDT sessions and obtaining cytologic preparations from lymph nodes were described in detail in the previously published article [1].

Cytological examinations were performed on preparations obtained by imprinting of irradiated and non-irradiated parts of lymph nodes on glasses.

The preparations were Romanowsky-Giemsa stained and microscoped under immersion (x1000) in transmitted light. The frequency of ill-defined cells (nuclei) was found by counting 500–1000 tumour cells and expressed as a percentage.

Statistical processing of data was performed with nonparametric techniques (Wilcoxon-Mann-Whitney U test) using the Statistica 13.3 software package.

Results

The findings of the study of 40 lymph nodes obtained by the above mentioned method were set forth in the previous article. Metastatic cells were detected in 23 lymph nodes; no metastatic cells were detected in 17 of them. Using DNA electrophoresis, apoptosis (apoptotic ladders) was detected in 17 of 23 lymph nodes affected by metastases [1].

Now we can confirm that cytological examinations in imprint smears performed in the same 17 lymph nodes revealed apoptotic bodies (Fig. 1).

During microscopy under immersion, a sharp contrast in the frequency of the so-called «bare nuclei» (BN) of malignant metastatic cells was found between irradiated and non-irradiated halves of lymph nodes. This phenomenon was observed both in squamous and glandular malignant tumours. In both cases, the nuclei had clear, even boundaries; the nature of staining and chromatin structure corresponded to intact cells with a cytoplasm and membrane (Fig. 2a). The observed «bare nuclei» were both located separately (Fig. 2b) and associated in the form of bunches of grapes, which probably corresponded to the induction of «bare nuclei» simultaneously and immediately after irradiation of metastatic cell complexes (Fig. 2).

Analyses of the frequency of BN of metastatic cells in irradiated and non-irradiated lymph nodes with metastases in 15 patients with adenocarcinoma and 19 patients with squamous cell carcinoma were performed in

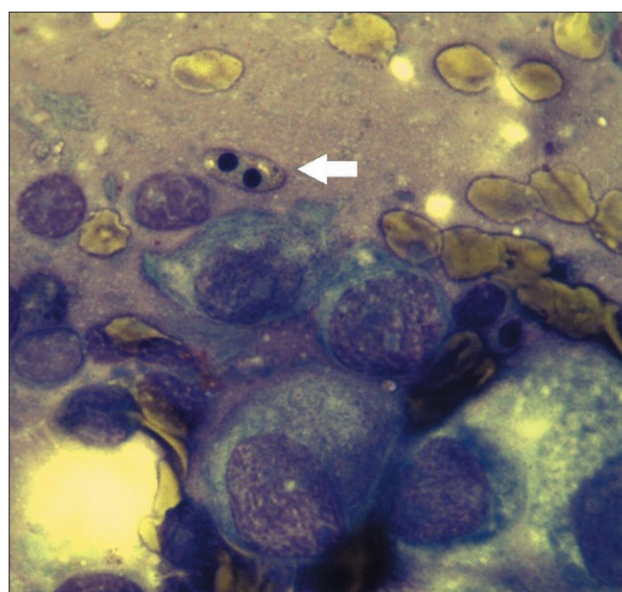


Рис. 1. Апоптотические тельца метастатических клеток аденокарциномы желудка в лимфатических узлах после проведения сеанса ФДТ

Fig. 1. Apoptotic bodies of metastatic cells in the lymph nodes of adenocarcinoma of stomach after PDT session

the STATISTICA programme and presented in bar graphs (Fig. 3, 4).

Obviously, the effect of IOPDT significantly increases the number of «bare nuclei» ($p < 0.0001$, Wilcoxon-Mann-Whitney U test).

Thus, we obtained cytological validation of apoptosis (the presence of apoptotic bodies) in lymph nodes with metastases after IOPDT, which we had previously detected using DNA electrophoresis [1]. Moreover, in our opinion, the most important thing revealed during this study was the «under the beam» effect manifested in the form of induction of «bare nuclei» of metastatic cells after PDT.

Cytological evidence of the death of tumour cells by apoptosis and necrosis allows us to conclude that IOPDT can increase the ablaticity of surgical interventions and improve the oncological outcomes of resections for locally advanced malignant tumours of the gastrointestinal tract.

Discussion

Obviously, the observed PDT-induced «bare nuclei» could only occur when the cells were damaged in the cytoplasm (lysosomes, mitochondria, Goldgi complex and endoplasmic reticulum) and cytoplasmic membrane, but not inside or even on the surface of the cell nucleus. Nucleus intactness during PDT was observed in the study of the subcellular localization of Foscan in the MCF-7 human adenocarcinoma line by M. H. Teiten et al. [2]. Using confocal microscopy and microspectrofluorimetry, the authors showed that this PS slightly accumulates in lysosomes and mitochondria and is mainly localized in the Goldgi complex and endoplasmic reticulum, without affecting the nucleus. According to the study by A. P. Castano et al., the PSs are localized in mitochondria, lysosomes, endoplasmic reticulum, Goldgi

complex and plasmalemma [3]. S. Farrakhova et al. studied the localization of chlorine e_6 and its dimethyl ether in HT29 cells of the human adenocarcinoma and found that these photosensitizers are mainly distributed in the plasmalemma and cytoplasm of cells and scarcely accumulate in the area of cell nucleus localization [4].

However, L. S. Fontana et al. obtained ambiguous results when studying the intracellular localization of the fotoditazine PS in the 9L/LacZ glioblastoma cell line. Fluorescence microscopy showed diffuse uptake of the PS in the whole cell, but it was impossible to say for certain whether the PS accumulates in the nucleus. The author himself believes that the false-positive result could be related to the association of fotoditazin with the nuclear membrane [5]. L. S. Fontana also faced the phenomenon of induction of «bare nuclei» and gave them a description that is entirely congruent with the one we had previously presented [6]. The very first report on the induction of BN during PDT, which we managed to find in the PubMed search engine, is given in the Chinese journal by the researcher Y. T. Zhou et al. [7]. It should be noted that «bare nuclei» of L. S. Fontana and «bare nuclei» of Y. T. Zhou were induced by PDT in cell cultures *in vitro*.

Despite the fact that L. S. Fontana's data indicate the association of the PS with the nucleus, it is obvious that the presence of radahlorin on the nucleus surface is still insufficient for its destruction after photoactivation.

The damage radius of the activated PS is relatively small on a subcellular scale [2, 3, 8, 9]. High reactivity and a short half-life of the singlet oxygen and hydroxyl radicals directly affect only molecules and structures that are located close to the area of their production (PS localization area). The half-life of singlet oxygen in biosystems is < 40 ns, therefore the radius of action of singlet oxygen is

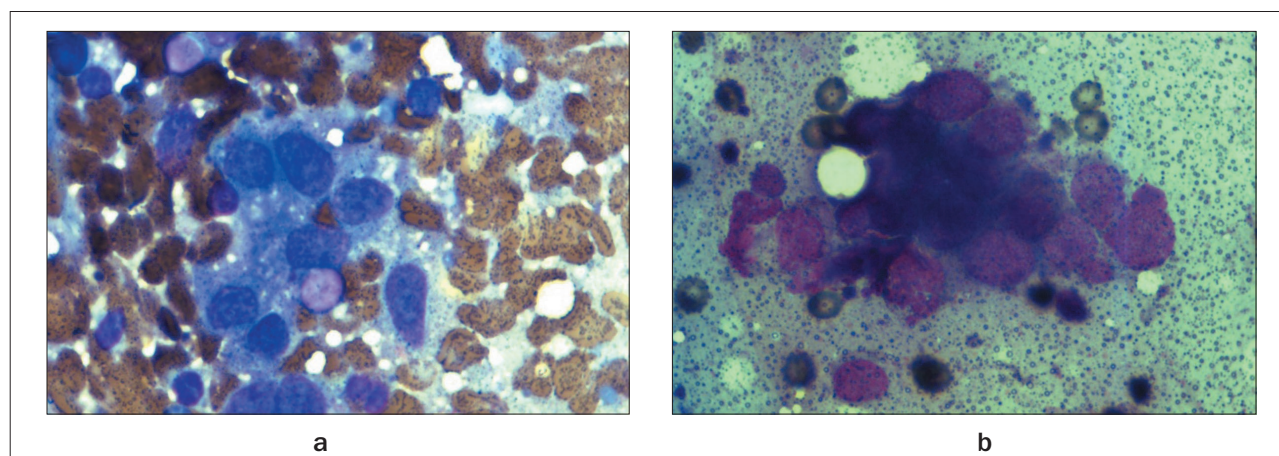


Рис. 2. Мазки-отпечатки метастатических клеток в лимфатическом узле 9А зоны абдоминальной лимфодиссекции до облучения (а) и после завершения (б) сеанса интраоперационной фотодинамической терапии, выполняемой аппаратом «Фара-2» в течении 20 мин (окрашивание азур-эозином по Романовскому, увеличение: $\times 1000$)

Fig. 2. Imprint smear of metastatic cells in the lymph node (9A) of the abdominal lymph node dissection zone before (a) and after (b) intraoperative PDT session carried out using «Fara-2» device for 20 minutes (staining with azur-eosin by Romanovsky, magnification: $\times 1000$).

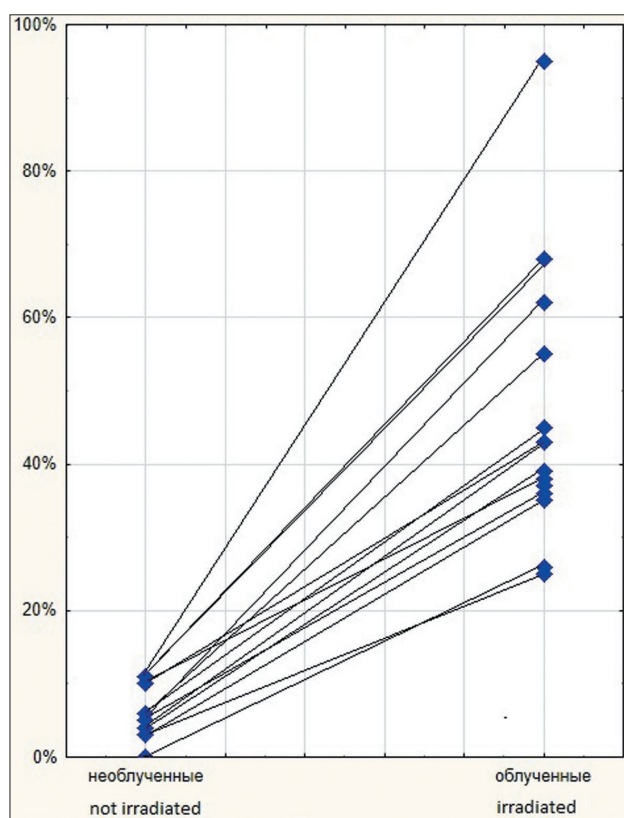


Рис. 3. Распределение половин лимфатических узлов по показателю «частота встречаемости "голых ядер"» при железистом раке

Fig. 3. Distribution of halves of lymph nodes according to the "frequency of occurrence of "naked nuclei" with glandular cancer

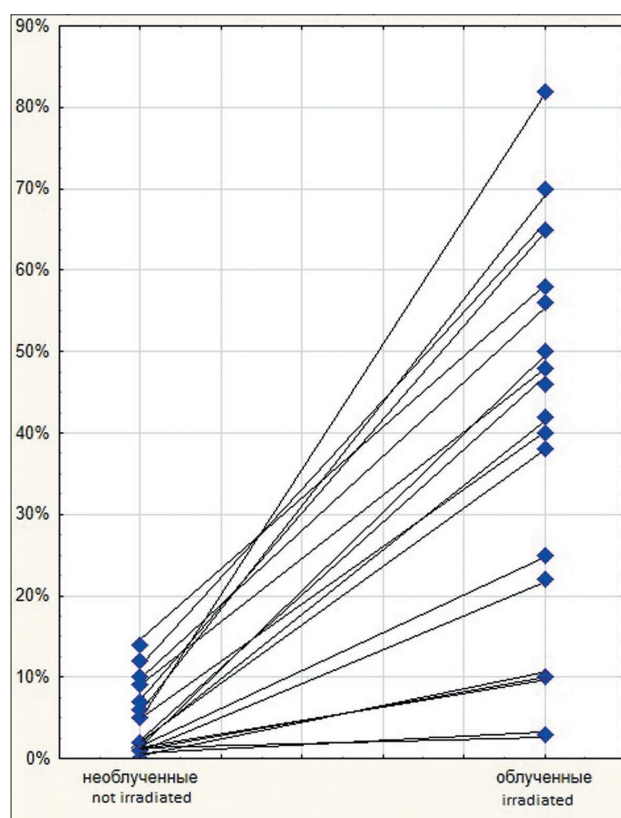


Рис. 4. Распределение половин лимфатических узлов по показателю «частота встречаемости "голых ядер"» при плоскоклеточном раке

Fig. 4. Distribution of halves of lymph nodes according to the "frequency of occurrence of "naked nuclei" with squamous cell carcinoma

about 20 nm [2], while the thickness of the nuclear membrane (34–74 nm) is greater by several fold than the possible damage radius of the photosensitizer. That is why the nucleus generally holds its shape after PDT.

Conclusion

IOPDT conducted in patients with malignant tumours of the gastrointestinal tract causes the «under the beam» effect, which is manifested in the induction of «bare nu-

clei» of metastatic cells of abdominal lymph nodes of the lymph node dissection area. During the study, cytological validation of apoptosis (the presence of apoptotic bodies) in lymph nodes with metastases after PDT sessions in the lymph nodes of the abdominal lymph node dissection area was presented. The use of IOPDT in a clinical setting increases the ablaticity of surgeries and oncological effectiveness of surgical treatment of locally advanced forms of the gastrointestinal cancer.

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