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CLINICAL AND MORPHOLOGICAL ASPECTS OF LOW-TEMPERATURE STORAGE OF MALIGNANT TUMORS

The article discusses the possibility of long-term low-temperature postoperative storage of malignant tumors for the purpose of manufacturing an individual anticancer vaccine. Morphological analysis of 23 fragments of malignant tumors (skin melanoma, colon cancer, breast cancer, soft tissue fibrosarcoma) was carried out at different temperature regimens (–20, –196 °C) and storage periods: 2-4 months, 10—12, 22—24 and 36 months. The percentage of preserved cells in the malignant tumors was determined in ten randomly selected fields of view on histological slides according to the method of G. Avtandilov. In skin melanoma samples stored at –20 °C, histologically intact tissues were (78.5 ± 4.1)%, and after storage in liquid nitrogen — (72.0 ± 5.1)%. The histological structure of colon cancer fragments under storage conditions at –20 °C was intact at (71.8 ± 5.3)%, and when stored in liquid nitrogen, regardless of the duration of cryopreservation, total necrosis was observed in two fragments, and in two more fragments the preserved tumor parenchyma was 63.3 and 90.8%, respectively. In breast cancer fragments stored at –20 °C, morphologically intact tissues constituted (73.1 ± 5.1)%. Soft tissue fibrosarcoma fragments during long-term storage at –20 °C consisted of (82.0 ± 3.6)% morphologically unchanged structures. Based on the results of this study and relevant scientific publications, the theoretical aspects of the production of personalized anticancer DC vaccines or mRNA vaccines, the antigenic substrate for which can be samples of malignant tumors stored at low (–20 °C) and ultra-low (–196 °C) temperatures, have been substantiated.

Key words: *malignant tumors, cryopreservation, tumor morphology, anticancer vaccines.*

The main methods of treatment of patients with malignant tumors are surgery, cytostatic chemotherapy and radiotherapy in various combinations. In recent decades, targeted drugs and immunotherapy with immune checkpoint inhibitors have been actively used, significantly improving the treatment outcomes in cancer patients. However, as shown by the results of clinical observations, the use of these drugs is also unable to solve the challenges of treating cancer patients in stage III—IV [20, 26, 27]. For such patients, only temporary efficacy of anticancer treatments can be predicted at the initial examination. If regional lymph nodes are also affected by metastases, the possibility of cure is halved [26]. What can be offered to such patients in anticipation of relapse or distant metastases? Among the available methods of cancer immunotherapy, personalized vaccines may be an option.

Vaccine therapy is one of the methods of treating cancer patients with a high risk of developing metastatic disease, aimed at inducing a long-term immune response in the patient to suppress and prevent the development of relapses and metastases [12, 18]. Now, the use of messenger RNA (mRNA) is considered a promising technology for creating

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personalized vaccines. The development of a vaccine against COVID-19 has opened up new ways for the application of mRNA technology to create personalized cancer vaccines [5, 29].

The R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR, Kyiv) has developed a technology for producing an autovaccine from the patient's own tumor and products of microbial synthesis of *Bacillus subtilis* [17]. The National Cancer Institute (Kyiv) uses a method for obtaining an individual antitumor vaccine based on dendritic cells (DC). The use of DC autovaccine in patients with non-small cell lung cancer increased the overall 5-year survival of these patients by 25% [15]. However, such technologies for manufacturing autovaccines require delivery of fragments of a "fresh" malignant tumor to the laboratory directly from the operating room, which limits the use of vaccine therapy in patients treated in Oncology Hospitals in other cities. Considering that only a few specialized laboratories are engaged in the manufacture of vaccines, the task of postoperative low-temperature storage of malignant tumor fragments becomes relevant. This would create the possibility of delivering tumor samples (in fact tumor antigens) from any remote hospital to specialized laboratories even several weeks or months after surgery. The first step in this process belongs to the surgeon, who, by removing a prognostically unfavorable malignant tumor, can preserve a tumor fragment or a large metastatic lymph node as a substrate for the manufacture of a personalized anti-cancer vaccine in the distant postoperative period.

The aim of the work was to investigate morphological changes (histological structure) of malignant tumor fragments after their prolonged low-temperature storage.

MATERIALS AND METHODS

The object of the study was malignant tumors stored at low (–20 °C) and ultra-low temperatures (–196 °C). The subject of this study was histostructural changes in the parenchyma and stroma of tumors and clinical and morphological substantiation of the possibility of using frozen samples of malignant tumors as a substrate for the production of individual anticancer vaccines.

The retrospective study involved medical records and histological specimens of 13 patients with malignant tumors of stages II—III. Patients underwent radical surgical interventions in 2016—2018: 5 for skin melanoma (3 men, 2 women), 4 for colon cancer (2 men, 2 women), 3 for breast cancer (3 women), 1 for soft tissue fibrosarcoma (1 man). The patients' ages ranged from 40 to 75 years. All patients were treated at the Ternopil Regional Clinical Oncology Dispensary with the signing of the relevant informed consent. The procurement and research use of human biological material were carried out in compliance with the ethical standards of the Declaration of Helsinki of the World Medical Association "Ethical Principles of Medical Research Involving Human Subjects". The study design was approved by the Ethics Committee of the Ternopil National Medical University.

A total of 36 histological sections of malignant tumors were reviewed, among which 13 were "native" preparations that were made immediately after radical surgeries. In these patients, the tumors had a large volumetric mass (Fig. 1 and 4) without distant metastases at the time of surgery. Immediately after surgery, several tissue fragments were excised from each tumor for cryopreservation: skin melanoma (9 fragments), colon cancer (7 fragments), breast cancer (5 fragments), soft tissue fibrosarcoma (2 fragments). Thus, a retrospective morphological analysis was performed in 23 fragments of malignant tumors that were stored at –20 °C (15 fragments) and –196 °C (8 fragments) (Table).

The collection of biological material and storage of tumor fragments was carried out using the following method: fragments measuring 2.0 × 2.0 × × 2.0 cm were cut from the surgical tumor specimen, dried several times with filter paper, then placed in a refrigerator (4—8 °C) for 30—40 min. After cold equilibration, tumor fragments in plastic containers were transferred to a freezer (–20 °C) for long-term storage.

To store tumor fragments at ultralow temperature (–196 °C), they were first placed in plastic bags, filled with cryoprotectant solution and transferred to a refrigerator (4—8 °C) for 3 hours. Then the bags were transferred to a Dewar vessel for storage in liquid nitrogen. The cryoprotectant solution consisted of glicerol (10 ml), lactose (11.5 g) and distilled water (90 ml). This composition of the cryoprotectant is used at the Institute of Biomedical Technologies (Ternopil) for the production of cryolyophilized xenoderm grafts.

For histological examination, tumor fragments stored at –20 °C were thawed at room temperature

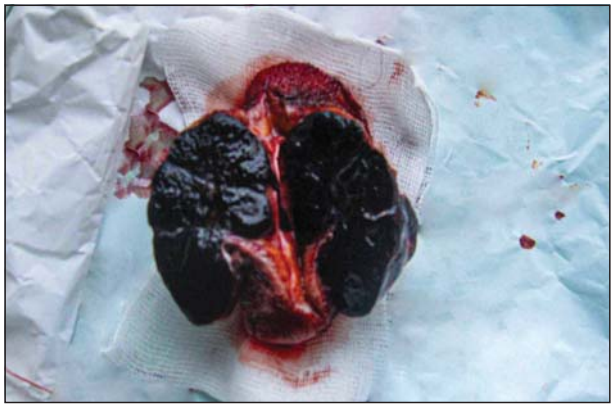

Fig. 1. Sectioned surgical specimen of skin melanoma. Exophytic tumor measuring 6 × 8 × 7 cm with invasion of all layers of the dermis

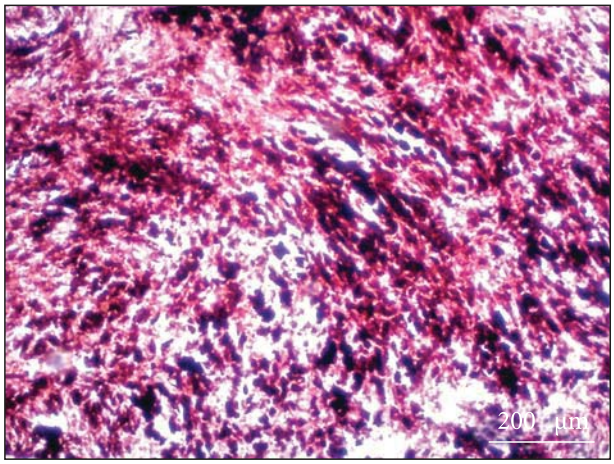

Fig. 2. Histological examination of skin melanoma ("native" histological slide 24678–9): in the deep layers of the dermis the infiltrative growth of melanoma is pronounced, melanocytes are oversaturated with pigment. Hematoxylin and eosin staining

(18—22 °C) for 4 hours; tumor fragments stored at –196 °C were thawed for 7—8 hours at 18—22 °C. Thawed tumor fragments were fixed in 10% neutral formalin solution with subsequent preparation of histological slides according to the standard techniques. Microslides were stained with hematoxylin and eosin. In a light-optical microscope "Olympus CX21FS2" (Olympus Corporation, Philippines), the percentage of intact structures in the parenchyma and stroma of the tumor was determined according to the method of G. Avtandilov [8, 21]. For this purpose, a morphometric grid consisting of 100 equidistant points was placed in the eyepiece of the microscope. In 10 randomly selected fields of view in each microslide, the tissue architecture of the tumor, the structure of cells and their nuclei, and the state of the stroma were evaluated. In each field of view, we determined the relative area of tissue where the typical structure of a malignant tumor was preserved. The average percentage was calcu-lated for each microslide. The same method was used to examine microslides of "native" tumors, which were not exposed to low temperatures, since the histological blocks were prepared immediately after surgery.

The changes considered as histological markers of apoptosis were as follows: reduction in cell size and karyorrhexis while maintaining the integrity of the basement membrane, the appearance of apoptotic bodies, the absence of a perifocal inflammatory reaction. The development of parabiosis and necrosis was evidenced by: nuclear hypochromatosis, karyopyknosis and karyolysis, cytoplasmic condensation, intracellular edema with subsequent cytolysis and cell fragmentation, disorganization of fibrous structures and inflammatory reaction in the stroma.

Descriptive statistics were used for statistical analysis; the results were expressed as a percentage of preserved morphological structure of the tumor.

RESULTS AND DISCUSSION

In "native" tumor slides, cells showing signs of apoptosis, destruction, or necrosis accounted for 4—10%, *i. e.,* the intrinsic morphological structure of malignant tumors in postoperative histological sections was preserved at 90—96%.

Morphological characteristics of cutaneous melanoma. Postoperative histological examination was performed in 5 large nodular melanomas (Fig. 1).

In "native" histological sections, the cellular component of melanoma was up to 90%, the area of stromal fibrous-fatty structures was up to 10%. Lymphocytic infiltration was weakly or moderately pronounced. Moderate stromal edema was noted. In the examined fields of view, 92—96% of melanoma cells were preserved (Fig. 2).

The effect of low and ultralow temperatures on histological structure of skin melanoma was evaluated in 5 tumor fragments stored at –20 °C and in 4 melanoma fragments stored at –196 °C.

The histological architecture of skin melanoma fragments stored at –20 °C differed from histological sections of the "native" tumor in that, against the background of stromal edema, approximately 15—30% of melanoma cells showed pronounced intracellular edema, vacuolization, and signs of

nuclear karyorrhexis (Fig. 3, *a*). Regardless of cryopreservation duration, 70—85% (on average (78.3 ± ± 4.1)%) of skin melanoma tissues had a morphological structure specific to this tumor.

Results of histological examination of 4 fragments of skin melanoma after storage at ultralow temperatures (–196 °C) showed that morphological structure of the tumor was preserved by 64—78% (on average (72.0 ± 5.1)%). Cryodamage was manifested either by rupture or deformation of cytoplasmic membranes, disruption of spatial organization of cells, karyolysis and pronounced edema of the stroma. In some places, alterative changes reached a critical level, as evidenced by the development of liquefactive necrosis (Fig. 3, *b*).

Morphological characteristics of colon cancer. Postoperative morphological study was performed in 4 large tumors that caused complete or subtotal colon obstruction (Fig. 4).

Histological sections of "native" colon tumors were characterized by infiltrative growth of adenocarcinoma in the muscle and subserosal layers. The tumors were represented by epithelial cells with signs of atypia, high mitotic activity and atypical mitoses, which formed tubular structures (their share in the total tumor tissue volume was > 95%) and small solid areas (Fig. 5). Lymphovascular invasion was absent (LVI–0). Peritumorally lymphomacrophage infiltration was observed. The proportion of preserved atypical epithelial cells in the carcinoma was 90—96%.

The effect of low and ultralow temperatures on histological structure of colon adenocarcinomas was evaluated in 3 tumor fragments stored at –20 °C and in 4 fragments stored at –196 °C.

The histological structure of tumors after storage at a temperature of –20 °C was characterized by moderate pathological changes, which, in essence, were an artifact of freezing and subsequent thawing: vacuolization, delamination and stretching of fibrous structures, disruption of nuclear morphology. At the same time, the architectonics of the tumor parenchyma was preserved by 72—80% (Fig. 6).

Among 4 fragments of colon cancers stored at –196 °C, total cryonecrosis was observed in 2 fragments (after 2 and 22 months), which was manifested by the loss of glandular tumor structure, destruction of nuclear components, fragmentation of membranes, and pronounced cellular edema of the stroma (Fig. 7, *a*). In the other 2 fragments (2 and 12 months of storage), the histological archi-

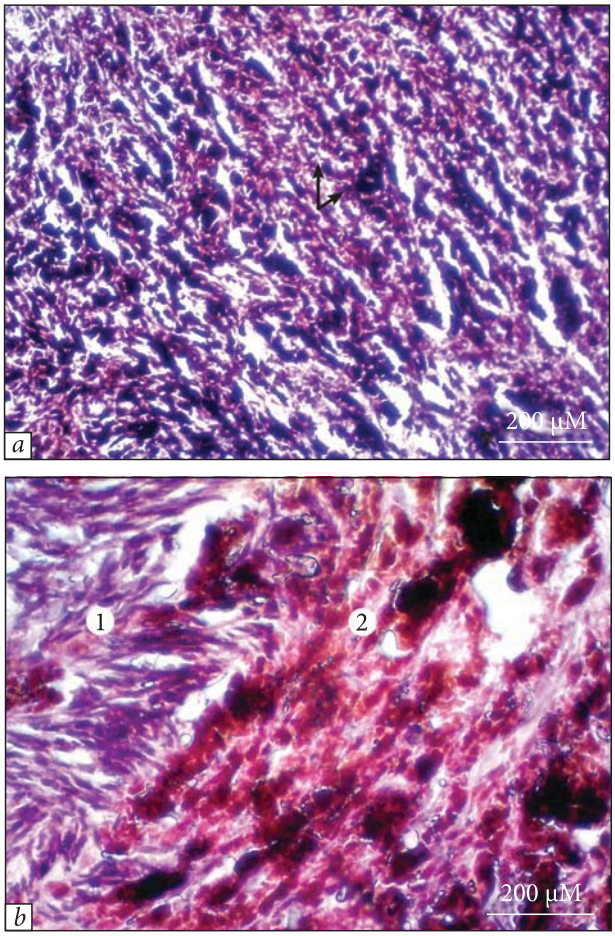

Fig. 3. Histological studies of skin melanoma: *a* — after 2 months of storage at –20 °C (histological slide 2092-4, the arrow shows intracellular edema of melanoma cells and stromal edema); *b* — after 22 months of storage at –196 °C (histological slide 25135-6, stromal edema, connective tissue fission (1), karyorrhexis (2), intracellular necrobiosis). Hematoxylin and eosin staining

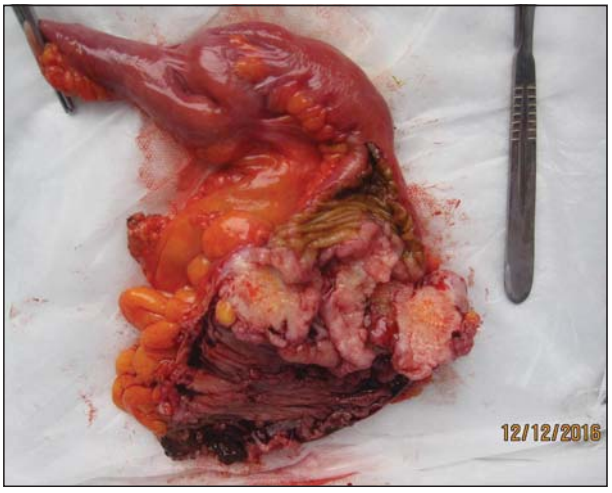

Fig. 4. Surgical specimen after a right-sided hemicolectomy for ascending colon cancer

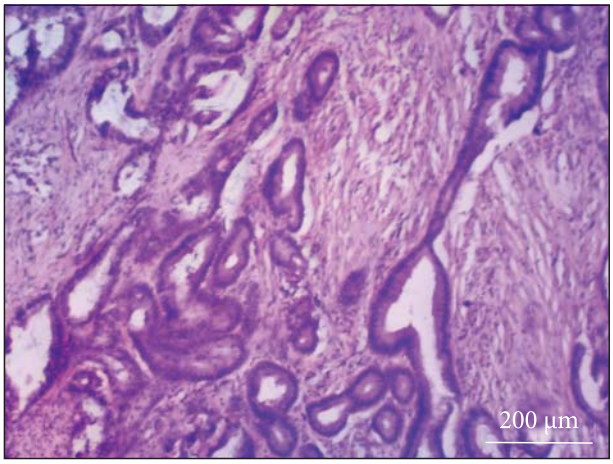

Fig. 5. Histological examination of a colon cancer ("native" histological slide 26306-14): infiltrative growth of highly differentiated adenocarcinoma in the muscle layer, moderate lymphocytic infiltration. Hematoxylin and eosin staining

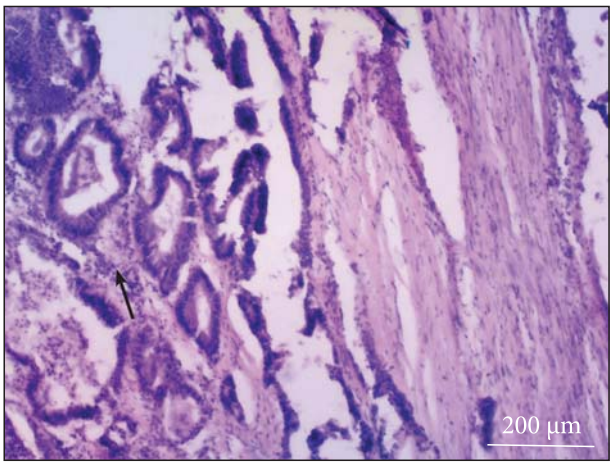

Fig. 6. Histological examination of a colon cancer after two months of storage at –20 °C (microslide 2098-00): the architectonics of adenocarcinoma is preserved by 80%, the tumor extends to 2/3 of the muscle layer with pronounced stromal edema and lymphocytic infiltration, shown by the arrow. Hematoxylin and eosin staining

tecture of adenocarcinoma was preserved by (90.8 ± ± 4.2)% (Fig. 7, *b*) and (63.3 ± 6.3)%. The Fig. 7, *b* shows a histological examination of a colon tumor fragment after 2 months of storage at –196 °C: the morphological structure of adenocarcinoma is identical to the "native" postoperative histological preparation.

Morphological characteristics of breast cancer. Postoperative morphological study was performed in 3 tumors that were removed radically. The architecture of "native" breast carcinomas was characterized by signs of invasive growth and consisted of atypical epithelial cells of various shapes and sizes with moderate nuclear atypia and moderate mitotic activity. The cells formed glandular, scirrhous structures, small solid areas, which was relevant for moderately differentiated (Grade 2) carcinomas. Focal lymphomacrophage infiltration was noted around the tumor growths. The histological integrity of the tumors was (91.2 ± 6.4)% (Fig. 8, *a*).

The effect of temperature factor was evaluated in 5 fragments of breast tumors stored at –20 °C. The tissue structure of tumors after cold storage from 2 to 24 months underwent moderate changes: edema and disorganization of fibrous elements of the desmoplastic stroma, intracellular edema and vacuolization of atypical epithelial cells in glandular and scirrhous structures, manifestations of karyorrhexis in some of them (fig. 8, *b*). At the same time, the level of preservation of the tumor parenchyma was (64.4—81.8)% (on average (73.1 ± 5.1)%).

Morphological characteristics of soft tissue fibrosarcoma. Tumor fragments were examined after surgical removal, as well as after storage at –20 °C for 2 and 12 months.

During histological examination of the postoperative material, a high-grade fibrosarcoma was detected, which was formed by markedly pleomor-

Distribution of tumor fragments (*n* = 23) by storage time and temperature regimens

Tumor fragments	Term and temperature regimens of storage of tumor fragments							
	2—4 months		10—12 months		22—24 months		36 months	
	–20 °C	–196 °C	–20 °C	–196 °C	–20 °C	–196 °C	–20 °C	–196 °C
CM	1	1	2	1	1	1	1	1
CRC	1	2	1	1	1	1	—	—
BC	1	—	2	—	2	—	—	—
STS	1	—	1	—	—	—	—	—

Notes: CM — cutaneous melanoma; CRC — colorectal cancer; BC — breast cancer; STS — soft tissue sarcoma (fibrosarcoma).

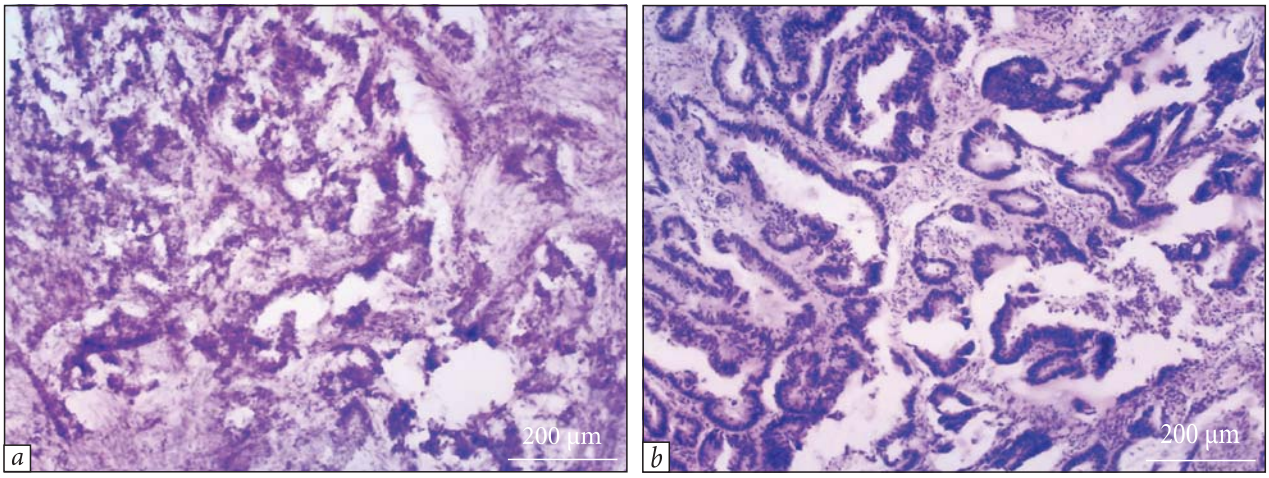

Fig. 7. Histological examination of colon cancer fragments after 2 months of storage at –196 °C: *a* — total cryonecrosis of the tumor (histological slide 2107-8); *b* — preserved histological structure of adenocarcinoma (histological slide 2103-4). Hematoxylin and eosin staining

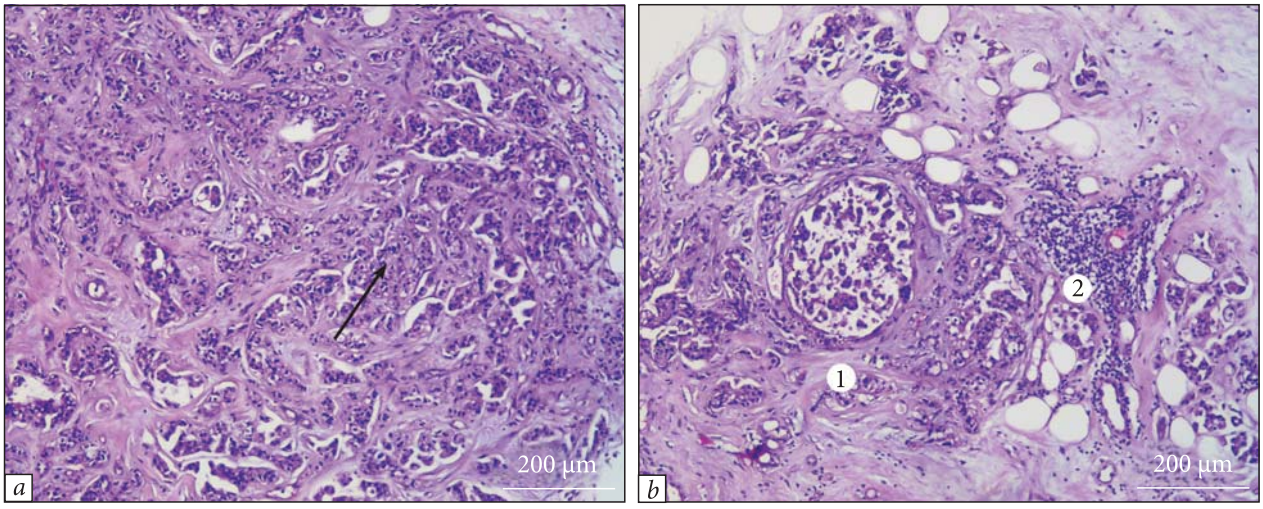

Fig. 8. Histological examination of breast carcinoma: *a* — immediately after mastectomy ("native" histological slide 19975-7, infiltrative growth of glandular cancer with the formation of scirrhous and glandular structures is indicated by an arrow); *b* — after 2 months of storage at –20 °C (histological slide 22482-4, architectonics moderately disturbed, stroma with edematous phenomena (1), lymphocytic infiltration moderately pronounced (2)). Hematoxylin and eosin staining

phic cells of spindle-shaped, polygonal and irregular shape, with signs of invasive growth. The nuclei were hyperchromatic, and numerous mitoses, including atypical forms, were visualized. The cells were arranged in chaotic clusters, occasionally forming trabecular and cribriform structures. The histostructural integrity of the tumor tissue was (94.0 ± 3.9)% (Fig. 9, *a*).

The tissue structure of soft tissue fibrosarcoma after cold storage at a temperature of –20 °C for 2 and 12 months underwent the following transformations: swelling and vacuolization of cells, lysis of cell membranes, edema and delamination of fibrous structures, individual foci of liquefactive necrosis (Fig. 9, *b*). At the same time, the integrity of the tumor parenchyma was (82.0 ± 3.6)%.

Cryobiological research of malignant tumors is relevant both in scientific and clinical aspects, especially in relation to the production of individual anti-cancer vaccines using frozen tumors. In what cases in clinical practice is it appropriate to consider cryopreserving a tumor or its fragments? First, in the presence of large primary tumors with a clinically foreseeable unfavorable prognosis; second, in cases of operable oligometastases that have arisen some time after removal of the primary tumor. Such patients always have a "window" of relapse-free period, which can last several months, and this

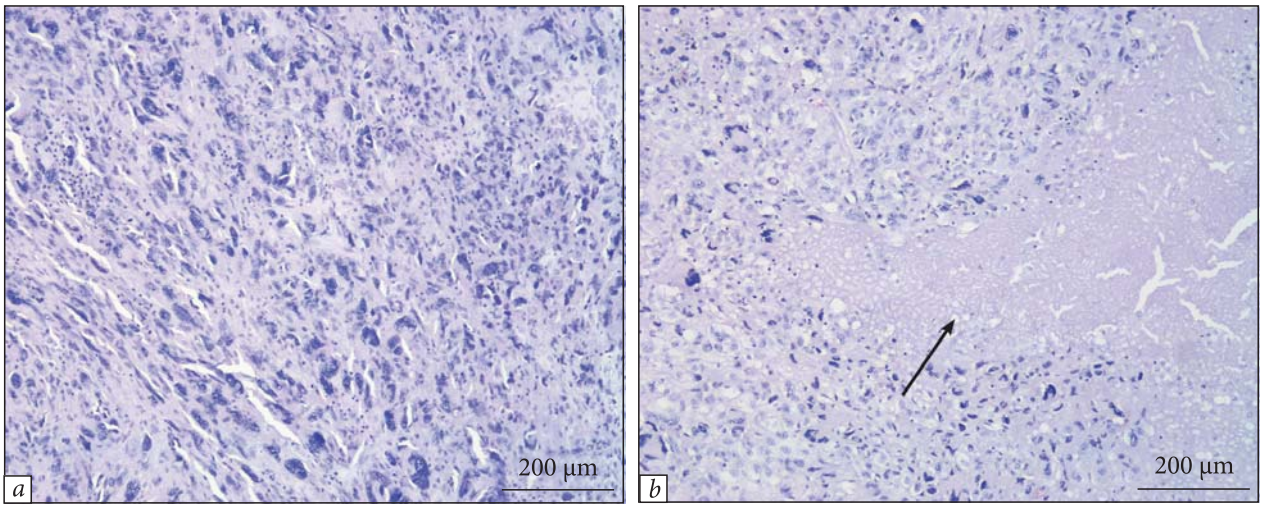

Fig. 9. Histological examination of soft tissue fibrosarcoma: *a* — postoperative "native" histological slide (21054-7): infiltrative tumor growth, pronounced cellular and nuclear atypism, formation of "moire" structures; *b* — after 2 months of storage at –20 °C (histological slide 24152-5, architectonics is disturbed, alterative changes in tumor cells, the focus of liquefactive necrosis is indicated by an arrow). Hematoxylin and eosin staining

time must be used to produce a personalized anticancer vaccine.

The DC and mRNA-based vaccines have proven themselves most in clinical practice. Dendritic cells are important for the induction of antitumor immunity and play a key role in anticancer immunotherapy by inducing $CD8^+$ T-cell immunity [12]. Adjuvant DC autovaccination in patients with cutaneous melanoma with operable metastases in regional lymph nodes improved overall survival by half: 63.6 months versus 31.0 months in the control group, and the 5-year survival rate was 53% in the vaccinated group of patients versus 38% in the control group [3].

Encouraging results have been reported with a number of DC vaccines, but such treatments still need to be optimized. Partial ineffectiveness of DC vaccination has been explained by the immunosuppressive tumor microenvironment, overexpression of checkpoint proteins, suboptimal avidity of specific T lymphocytes associated with tumor antigen, *etc*. Therefore, it is advisable to combine DC vaccination with immune checkpoint inhibitor therapy or targeted drugs [9, 20]. Positive results of DC vaccination in combination with ipilimumab have been obtained in patients with cutaneous melanoma with an objective response rate of 38% [4, 11].

In recent years, neoantigen vaccines have attracted considerable interest. Neoantigens are produced by cancer cells as a result of tumor-specific changes such as genomic mutation, unregulated RNA splicing, disordered post-translational modification, *etc*. The neoantigens are recognized as nonself and induce an immune response. Rapid identification of tumor-specific neoantigens has become possible with the progressive development of next-generation sequencing and bioinformatics technologies, including artificial intelligence. Compared with tumor-associated antigens, highly immunogenic and tumor-specific neoantigens provide new targets for personalized cancer immunotherapy [7, 28]. The technology is based on targeting tumor-specific mutations that are absent in normal cells. Each patient's tumor has a unique set of mutations. Therefore, the mutational fingerprint of the cancer can be used to develop personalized mRNA-based neoantigen vaccines. Artificial intelligence algorithms can rapidly analyze massive genomic data sets and help identify cancer mutations among the many genetic variations in a patient's tumor [23, 24].

For example, the mRNA-4157 (V940) vaccine targets tumor neoantigens that are expressed by tumors in a specific patient. The vaccine encodes up to 34 neoantigens and activates an antitumor immune response based on the patient's individual cancer mutations. To personalize it, a tumor sample is removed during surgery, followed by RNA sequencing to identify single-nucleotide variants that are present only in the tumor but absent in normal tissue. The result is a personalized vaccine. In a phase II clinical trial, mRNA-4157 (V940) vaccine in combination with an immune checkpoint inhibitor (Keytruda) was found to reduce the risk

of relapse or death by 44% compared with Keytruda monotherapy in patients with stage III/IV cutaneous melanoma. At the same time, the 18-month relapse-free survival was 79% in the combination therapy group, compared with 62% in monotherapy [27]. It can be argued that immunization of patients with RNA derived from autologous tumor cells is a promising immunotherapeutic approach.

New experimental studies have opened up another level of immunotherapy using small circular RNAs (circRNAs). The circRNAs encoding tumor-associated antigens and neoantigens have now been shown to elicit potent and sustained T-cell responses for robust antitumor immunotherapy, especially when combined with immune checkpoint inhibition. Compared with unmodified or modified mRNAs encoding large proteins, circRNA vaccines induced up to a 10-fold increase in the number of antigen-specific T cells in mice, which accounted for 30—75% of the total peripheral $CD8^+$ T cells during a 6-month follow-up [19, 30].

In the above-described studies, the "fresh" tumor fragments were taken immediately after surgery to produce autovaccines, while we propose using frozen tumor tissue. For this purpose, it is necessary to evaluate not only the preservation of tumor morphological structure and its antigenic activity, but also cryoimmunological aspects related to temperature regimens and storage duration, and morphological architectonics of different types of malignant tumors.

Factors affecting the preservation of cells after cryopreservation include the type and concentration of cryoprotectant, the freezing and thawing rate of biological objects, storage temperature regimens, *etc*. [1]. However, it is impossible to propose any optimal specific freezing and thawing technique for a wide range of tissues, taking into account their different architecture, density, and response to low-temperature storage regimens [16]. In our study involving 8 tumor fragments (4 — CRC, 4 — CM) stored at –196 °C, two CRC micropreparations showed total cryonecrosis, which was associated both with different architecture of these malignant tumors and the use of a cryoprotectant based on natural compounds with no addition of dimethyl sulfoxide.

There are known experimental studies which used repeated cycles of freezing and thawing, ultraviolet irradiation, tumor heating up to 42 °C, hypochlorous acid (HOCl) and other methods to obtain a whole-cell tumor lysate with increased expression of tumor antigens [6, 10]. The phenomenon of cryogenic protein denaturation is an important mechanism of cryogenic tissue damage after freeze-thawing. Cryocoagulated (or cryomodified) protein components, DCs, and cryocellular debris formed in living biological tissue after exposure to ultralow temperatures *in vivo* initiate the mechanisms of cryoimmune response of living structures to low temperature effects [16, 22]. In our series of studies, we observed the phenomena of partial cryogenic necrosis in the majority of tumor samples. Cryonecrosis of a part of the tumor during low-temperature storage may unexpectedly even be useful, assuming that fragmented cellular and stromal structures with the formation of corresponding protein molecules can be detected as neoantigens during sequencing.

Malignant tumor cells and tissues respond differently to low and ultralow temperatures. K. Santucci *et al.* [25] studied the response of the lung adenocarcinoma cell line A549 to freezing. It was found that a single 5-minute freezing to –15 °C did not affect cell viability, while freezing the cell line to –20 and –25 °C significantly reduced (< 10 %) their viability. J.M. Baust *et al.* [2] studied the *in vitro* response of a pancreatic adenocarcinoma cell line to freezing. The authors showed that freezing to –10 °C had no effect on viability, whereas temperatures of –15 and –20 °C reduced the percentage of viable malignant cells to 85 and 20%, respectively. Complete loss of cell structure was observed after a single freezing below –25 °C.

Therefore, according to the literature data [2, 25], the temperature of –20 °C is the limit for the viability of malignant tumor cell lines. Unlike cell lines, in our experiment, tumor tissue fragments were frozen at –20 °C without the use of cryoprotectants. At the same time, the preserved histological structure of skin melanoma, CRC, BC and STS was 62.2—85.5%, which depended on the features of morphological architectonics of each tumor. A significant percentage of tumor integrity preservation allows us to state with high probability that the antigenic activity of these tumors is also preserved, which is important for the production of personalized vaccines.

An improved method for cryopreservation (to –80 °C) of viable lung tissue was presented by J. Baatz *et al.* [1]. In this case, fresh and thawed lung tissue had minimal histological differences, and the

integrity of RNA and proteins was also not different. After three months of cryopreservation, numerous cell types were obtained from lung tissue, including alveolar epithelial cells, fibroblasts, and stem cells. In a study by X. Huang *et al.* [13], it was proven that programmed controlled slow freezing preserved the reversible viability of tissues after thawing, in contrast to flash freezing with liquid nitrogen. At the same time, the integrity of RNA, proteins and skeletal muscle microstructure is preserved. Slow programmed freezing is used by biobanks as a cryopreservation method.

The Ontario Tumour Bank has been conducting annual assessments of frozen samples for RNA and DNA integrity as part of ongoing quality control. This provided a unique opportunity to study the effects of long-term (2—11 years) storage of tissues in liquid nitrogen. Studies of thawed samples have shown that the quality of tissue RNA or DNA did not decrease throughout the entire storage period at ultra-low temperatures [14].

Thus, the data of the above-described scientific reports and the results of our studies prove the possibility of long-term and safe storage of tumors at low temperatures with preservation of their histological structure, and, consequently, their antigenic properties. Cryopreserved fragments of malignant tumors can be used for the production of neoantigenic and DC autovaccines in the remote postoperative period. For clinical practice, long-term storage of tumors at –20 °C is more advantageous with minimal economic costs, compared to storage at ultralow temperatures (–196 °C). Further clinical and laboratory studies will be aimed at studying the antigenic activity of frozen tumors and realizing the possibility of manufacturing individual anticancer DC or mRNA vaccines using samples of malignant tumors after their storage at low temperatures.

CONCLUSIONS

In our series of studies in "native" tumor slides, cells showing signs of apoptosis, destruction, or necrosis accounted for 4—10%; thus, the intrinsic morphological structure of malignant tumors in postoperative histological sections was preserved at 90—96%.

In samples of malignant tumors (cutaneous melanoma, colon cancer, breast cancer, soft tissue fibrosarcoma), which were stored from 2 to 36 months at a temperature of –20 °C, the percentage of their unchanged histological structure was 62.2—85.5%, which depended on the features of morphological architectonics of the tumor.

The integrity of morphological structure of malignant tumors stored for 2 to 22 months at –196 °C depends on the architectonics and density of the tumor tissue: in 4 fragments of skin melanoma, the percentage of cells with unchanged histological structure was (72.0 ± 5.1)%; among 4 fragments of colon adenocarcinoma, total cryonecrosis was observed in two, and in the other two fragments, the histological integrity of the tumor was 63.3 and 90.8%, respectively.

The preserved structure of malignant tumor tissues after cryopreservation is the biological substrate that can be used to produce individual vaccines based on their own tumor-associated antigens or neoantigens.

For long-term (several months) storage of malignant tumor fragments, it is sufficient to use the temperature regimen of the freezer (–20 °C).

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КЛІНІКО-МОРФОЛОГІЧНІ АСПЕКТИ НИЗЬКОТЕМПЕРАТУРНОГО ЗБЕРІГАННЯ ЗЛОЯКІСНИХ ПУХЛИН

У статті обговорюється можливість тривалого низькотемпературного післяопераційного зберігання злоякісних пухлин з метою виготовлення індивідуальної протиракової вакцини. Проведено морфологічний аналіз 23 фрагментів злоякісних пухлин (меланоми шкіри, раку товстої кишки, раку молочної залози, фібросаркоми м'яких тканин) при різних температурних режимах (–20, –196 °С) і термінах зберігання: 2—4 місяці, 10—12, 22—24 і 36 місяців. У мікропрепаратах пухлин визначали відсоток збережених клітин у десяти випадково обраних полях зору за методикою Г. Автанділова. У зразках меланоми шкіри, що зберігались при –20 °С, гістологічно цілісні тканини становили (78,5 ± 4,1) %, а після зберігання в рідкому азоті — (72,0 ± 5,1) %. Гістологічна структура фрагментів раку товстої кишки за умов зберігання при –20 °С була цілісною на (71,8 ± 5,3) %, а за зберігання в рідкому азоті, незалежно від тривалості кріоконсервування, у двох фрагментах спостерігався тотальний некроз, а ще у двох фрагментах збережена паренхіма пухлин становила 63,3 і 90,8 % відповідно. У фрагментах раку молочної залози, що зберігались при –20 °С, морфологічно цілісні тканини становили (73,1 ± ± 5,1) %. Фрагменти фібросаркоми м'яких тканин під час тривалого зберігання при –20 °С на (82,0 ± 3,6) % складались із морфологічно незмінених структур. На основі результатів даного дослідження і відповідних наукових публікацій обґрунтовано теоретичні аспекти виготовлення персоналізованих протипухлинних ДК-вакцин або мРНК-вакцин, антигенним субстратом для яких можуть стати зразки злоякісних пухлин, що зберігались при низьких (–20 °С) та ультранизьких (–196 °С) температурах.

Ключові слова: *злоякісні пухлини, кріоконсервування, морфологія пухлин, протиракові вакцини.*