The photodynamic therapy of transplanted colorectal carcinoma in normal and immunodeficient mice

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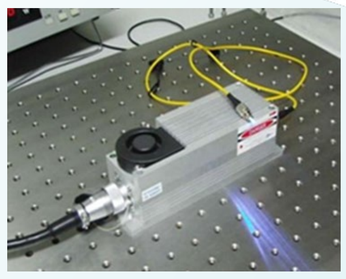
The aim of the study was to evaluate the effectiveness of photodynamic therapy (PDT) in normal and immunodeficient mice with transplanted colorectal carcinoma CT-26..

MATERIALS AND METHODS

Experimental protocol in vitro

Undifferentiated murine colon carcinoma cell line (CT26.WT) was used to induce subcutaneous tumor and cultured in DMEM supplemented with a 10 % FBS, and 1 % penicillin / streptomycin according to ATCC protocol before injection. Animal experiments were carried out using Balb/c and immunodeficient nu/j female mice (age, 7–8 weeks; number of animals, 16).. Cells were detached from the bottom of culture flask by means of incubation with Trypsin-EDTA for 7 minutes, collected, counted after Trypan Blue staining using Countess™ automated cell counter (Invitrogen) and centrifuged at 800 rcf for 5 minutes. Cell pellet was resuspended in appropriate volume of DMEM. A suspension of 0.25 million CT26.WT cells in 10 µL serum- and antibiotic-free DMEM was injected subcutaneously into the dorsal side of a left hind limb.

Photodynamic therapy of CT-26



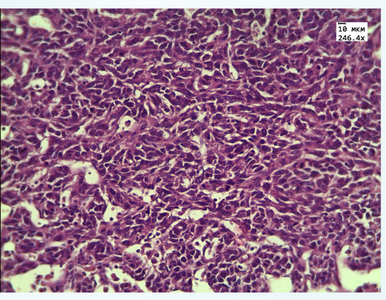
Experimental protocol in vivo

Balb/c and immunodeficient nu/j mice were injected intramuscularly with 20 µl of CT-26 colorectal cancer cell suspension. When the tumor volume reached 500 mm3, they were randomly divided into 2 groups - with photodynamic therapy and without any treatment.

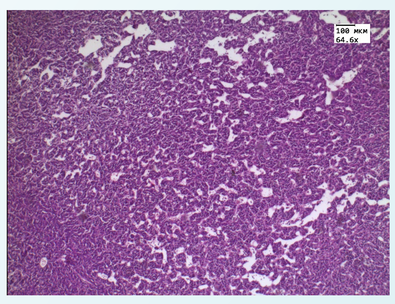
Indocyanine green was used for PDT, which was diluted in polyethylene glycol in a ratio of 1:100 and administered intravenously to mice at a dose of 2 mg/kg. One hour after intravenous administration, the tumors were irradiated with a percutaneous diode infrared laser with a wavelength of 808 nm at a power density of 2.3 W/cm2 for 10 minutes. The animals were euthanized by decapitation, the tumors were removed, and tumor tissues were collected for histological examination 72 hours and 21 days after therapy. Tumor samples were fixed in 10% formalin solution and embedded in paraffin. Histological sections were prepared according to common methods, followed by hematoxylin and eosin staining.

The immunohistochemical reactions with antibodies to proliferation marker Ki-67 and apoptosis marker BAX (Abcam, UK) were visualized with a REVEAL—Biotin-Free Polyvalent DAB kit (Spring Bioscience, USA). Morphological studies of the tumor tissue were performed using a Microvizor medical mVizo-103 (Russia).

Micrographs of tumor tissue in control group without any treatment - 72 hours



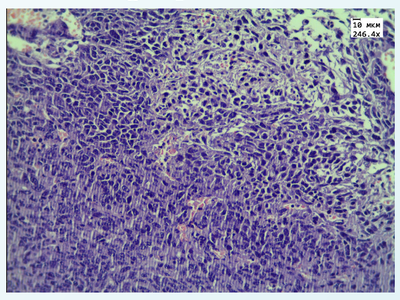
Balb/c mouse



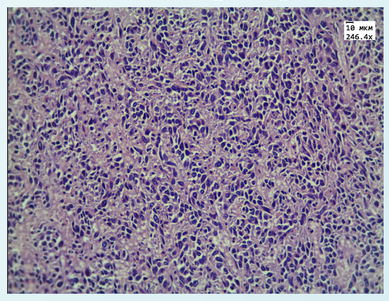
Immunodeficient mouse

Histological examination in control mice showed that the tumor is presented by polymorphic elongated atypical cells. The structure of tumor tissue did not differ between experimental groups of mice.

Micrographs of tumor tissue in control group – 21 days



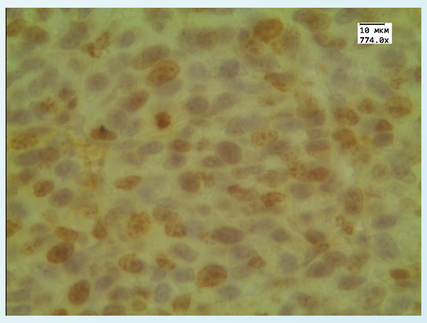
Balb/c mouse



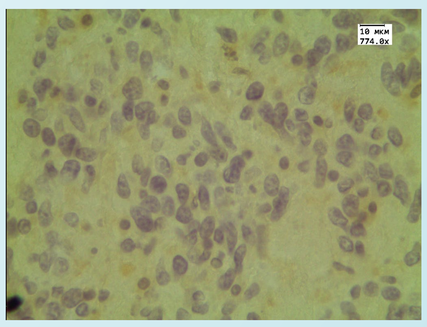
Immunodeficient mouse

The tumor volume increased to a greater extent in nudes, then in balb mice, however, the cell density in tumors of immunodeficient mice is reduced, areas of rarefaction caused by necrosis are determined, and tumor cells are arranged in chains.

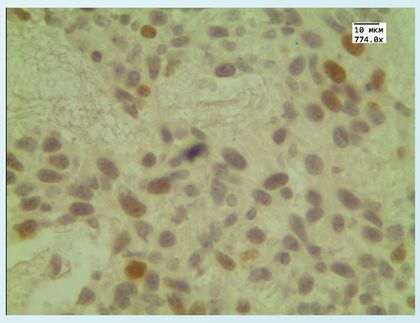
Immunohistochemical study (ICH) with antibodies to proliferation marker Ki-67 and apoptosis marker BAX revealed more pronounced expression of BAX and weaker expression of Ki-67 in tumor tissue 72 hours after PDT treatment. These changes in expression were more pronounced in tumors of Balb/c mice.



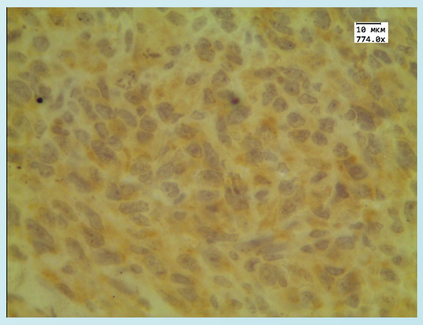
Without treatment, ICH with Ki-67



Without treatment, ICH with BAX



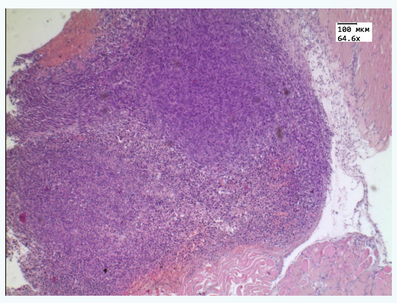
72 h after PDT, ICH with Ki-67



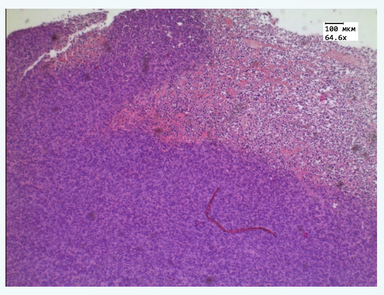
72 h after PDT, ICH with BAX

RESULTS

In PDT group 72 hours



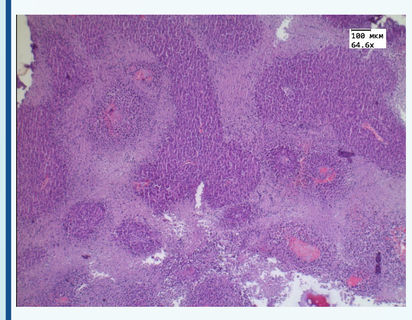
Balb/c mouse



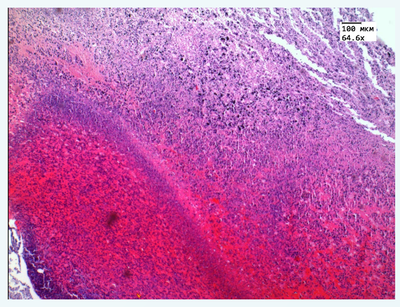
Immunodeficient mouse

Histological examination showed that 72 hours after PDT small areas of necrosis and hemorrhages were observed in the tumors

In PDT group 21 days



Balb/c mouse



Immunodeficient mouse

21 days after PDT, the inhibition of tumor growth was noted, which was more pronounced in Balb/c mice than in immunodeficient mice. The extensive necrosis and hemorrhages were observed in the tumors, necrosis fields occupied up to 70-80% of the area in tumors of Balb/c mice, the hemorrhages were more pronounced in nude mice.

Conclusion

The more pronounced inhibition of tumor growth after PDT in Balb/c mice compared to immunodeficient mice was revealed, probably due to the fact that PDT not only controls tumor growth by directly killing tumor cells, but also modulates the tumor microenvironment, promoting the attraction and activation of immune cells lacking in immunodeficient mice. This indicates the importance of evaluate the impact of PDT on immune system in cancer treatment.

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