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Biochemistry

The Effect of Microscopic Fungi on Cancer Growth

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(Presented by Academy Member Tinatin Sadunishvili)

Apoptosis is an important biological process that regulates the life and death of cells, plays a crucial role in maintaining tissue homeostasis. Dysregulation of apoptosis may lead to uncontrolled cell proliferation and tumor growth. In the present paper, the effect of microscopic fungi-derived biologically active substances: MII-12, NL-50 and Gz-9-10 on tumor growth and apoptotic processes in Ehrlich carcinoma-bearing mice have been studied. The anticancer effect of MII-12 has been shown. Cancer growth by means of this preparation was inhibited significantly as compared to control, NL-50 and Gz-9-10 treated animals. Morphological and biochemical examinations confirmed the anticancer properties of MII-12 biopreparation. Caspase-3 level was increased notably indicating stimulation of apoptosis. Morphological picture of cancer tissue showed large areas of necrosis. Microscopic fungi-derived biologically active substances may be considered promising for developing of innovative and targeted cancer therapies. *© 2024 Bull. Georg. Natl. Acad. Sci.*

cancer, microscopic fungi, apoptosis, caspase-3

Apoptosis, often referred to as programmed cell death, is a fundamental biological process that plays a crucial role in maintaining tissue homeostasis, preventing the proliferation of abnormal cells, and regulating cell populations within the body. Dysregulation of apoptosis is a hallmark of cancer, as it allows cancer cells to evade the body's natural defenses and grow uncontrollably [1, 2].

Apoptosis is a precisely regulated process involving a cascade of proteolytic events. Caspase-3, a member of the caspase family of proteases, plays a central role in the execution phase of apoptosis. Activation of caspase-3 is a key event in the apoptotic pathway, and it is often considered as a target for cancer therapy [3, 4].

Some biologically active substances isolated from microscopic fungi have been investigated for their ability to activate caspase-3 and induce apoptosis in cancer cells [5, 6].

Our previous studies have revealed that biologically active substances isolated from intracellular biomass/lysate obtained through solid-phase fermentation of microscopic fungi (Penicillium sp. Gz 9-10, Mucor sp. Gz II-12, Fusarium sp. NL 50) tend to inhibit tumor growth and increase the lifespan of tumor-bearing animals compared to control groups [7,8]. These findings suggest that the biologically active compounds isolated from microscopic fungi may possess antitumor activity, and one possible cause of this effect could be the activation of apoptosis.

In this work, the effect of biologically active compounds isolated from microscopic fungi on cancer growth and apoptosis has been studied. According to the results of the experiment it could be said that harnessing the power of caspase-3 to induce apoptosis in cancer cells is a compelling avenue in the ongoing quest for more effective and less toxic cancer treatments.

Materials and Methods

The 8- to 10-week-old male albino mice weighing 20-25 g were purchased from the vivarium of the A. Natishvili Institute of Morphology (Tbilisi, Georgia. https://www.tsu.ge/en). After being placed in a laboratory the animals were given a 7-day interval for acclimatization before the experiment. During this period the animals were maintained in a stable environment with a 12-hour light and 12-hour dark cycle. They were fed a standard laboratory chow and given free access to water; the temperature was consistently maintained at 23 ± 2°C. Experiments were approved by the Animal Research Ethics Committee of TSMU.

Disease modeling. The Ehrlich ascites carcinoma (EAC) cells were provided by the Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology of the National Academy of Sciences of Ukraine, Department of Experimental Cell Systems, The Cell Line Bank (BCL) from Human and Animal Tissues (https://iepor.org.ua/www.onconet.kiev.ua).

The EAC cells were propagated in our laboratory biweekly through intra-peritoneal injections of 5×10^6 cells/mouse. Cells were counted by the hemocytometer.

Under brief ether anesthesia, each mouse was inoculated subcutaneously in the right flank with a fixed number of viable cancer cells (2×10^6 cells/20 g body weight) freshly drawn from a donor mouse to induce the formation of a solid tumor. The viability of the EAC cells was 98% (by trypan blue exclusion assay).

Biopreparations. The Scientific and Educational Center "Biomed" at the Technical University of Georgia provided the intracellular lysates of microscopic fungi, acquired via solid-phase fermentation. Caspase 3 detection kits were purchased from Wuhan Fine Biotech Co., Ltd. albino male mice with a weight range of 20-25 g, from the local vivarium.

The animals were randomly divided into control and experimental groups (n = 8/group). To determine the influence of research samples (Mucor sp. Gz II-12, Fusarium sp. NL 50, Penicillium sp. Gz 9-10) the control group of mice received 100 µl physiological solution injections. The experimental group animals received injections of 100 µl of the test solution once a week, with a protein concentration of 2 mg/ml. The observation duration was 30 days.

Monitoring tumor volume. Throughout the research period, the study samples were assessed for their effects on tumor growth. Tumor volume was measured using a Vernier caliper on the 7th, 14th, 21st and 28th days after tumor transplantation. The tumor volume was calculated using the formula $V = a \times b^2 \times \pi/6$, where "V" is the mean tumor volume (cm^3), "a" – is the length, and "b" – is the width of tumor tissue [9].

Tissue collection and preparation for analysis. At the end of the experiment, all mice were euthanized under ether anesthesia. Tumor tissue was dissected out, and washed with cold PBS. For histopathological study, tissues were preserved in 10% formalin. As for biochemical research, samples were homogenized in a lysis buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 1 mM PMSF) and then centrifuged for 5 minutes at 5000×g, supernatants were frozen at 20°C for farther investigations.

Morphological examination. Tumor tissue was embedded in paraffin and cut into 5-µm slices. They were stained with Hematoxylin and Eosin.

Biochemical analysis. The effect of research samples on caspase 3 activity was detected using an ELISA assay kit following the manufacturer's instructions (Fine Test, Wuhan, Hubei, China). Test samples (100 µl each) were added to wells precoated with the capture antibody and incubated for 90 minutes at 37°C. After incubation, the wells were washed twice with wash buffer and then incubated with 100 µl of biotin-labeled working antibody for 60 minutes at 37°C. Following the removal of the antibody solution, the wells were washed again and incubated with 100 µl of SABC (HRP-streptavidin conjugate) working solution for 30 minutes at 37°C. The wells were washed five times, and 90 µl of TMB substrate was added. Incubation occurred for 10-20 minutes in a dark place. Afterward, a stop reagent was added, and the optical density was measured at 450 nm. The concentration of the Caspase 3 was calculated using a standard curve.

Statistics. SPSS software package (version 10.0) was used for analyzing data. Differences were considered significant at a level of $p < 0.05$.

Results and Discussion

Treatment of animals with experimental bioprepa-rations was started after one week of disease modeling when the mean tumor volume range was 0.9-1 cm^3. The results are presented in Figs. 1-3.

To some extent, the research samples exhibited an impact on inhibiting tumor growth. After the first week of administration, M II-12 samples showed a tendency of reduction in tumor growth by 24.4% compared to the control group animals. Tumor growth was significantly inhibited on the 2nd and 3rd weeks by 38.7% and 41%, respectively ($p < 0.001$).

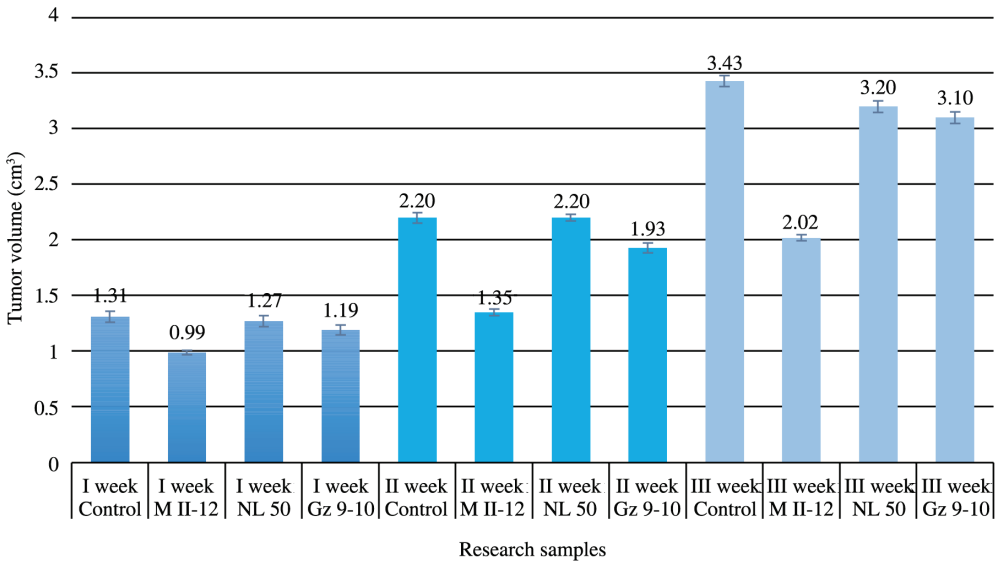

Fig. 1. Mean tumor volume (cm^3) after the treatment with M II-12, NL-50 and Gz-9-10.

In the case of treatment with NL 50 and Gz 9-10, the results of the experiment did not reveal the same antitumor effects of the biopreparations. There was not statistically significant difference in mean tumor volumes compared to control group animal data. In the case of NL 50 decrease in tumor volume by 3%, 0%, 6.7% during 1st, 2nd and 3rd weeks respectively, and in the case of Gz 9-10 decreased by 9.17 %, 12,3% and 9.63 % was not statistically significant.

Determination of Caspase-3 Level

Among caspases that play an essential role in programmed cell death or apoptosis caspase-3 is the frequently activated death protease, facilitating the targeted cleavage of numerous vital cellular proteins. Consequently, their level was assessed after exposing mice to research samples. The ELISA results showed that the caspase-3 level was increased under the influence of M II-12 compared to the control group. It was increased by 0.58 ng per milliliter. In the case of NL 50 and 9-10, a slight change was observed, which was not statistically significant (Fig. 2).

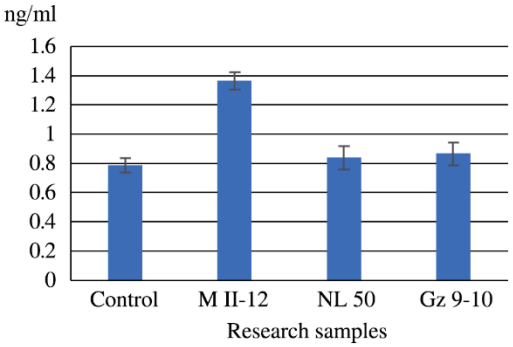

Fig. 2. Caspase-3 concentration in control and treated with M II-12, NL-50, and Gz-9-10.

Morphological picture N3 shows the Ehrlich carcinoma tissue of control and experimental animals treated with biopreparations on the 30th day of tumor growth.

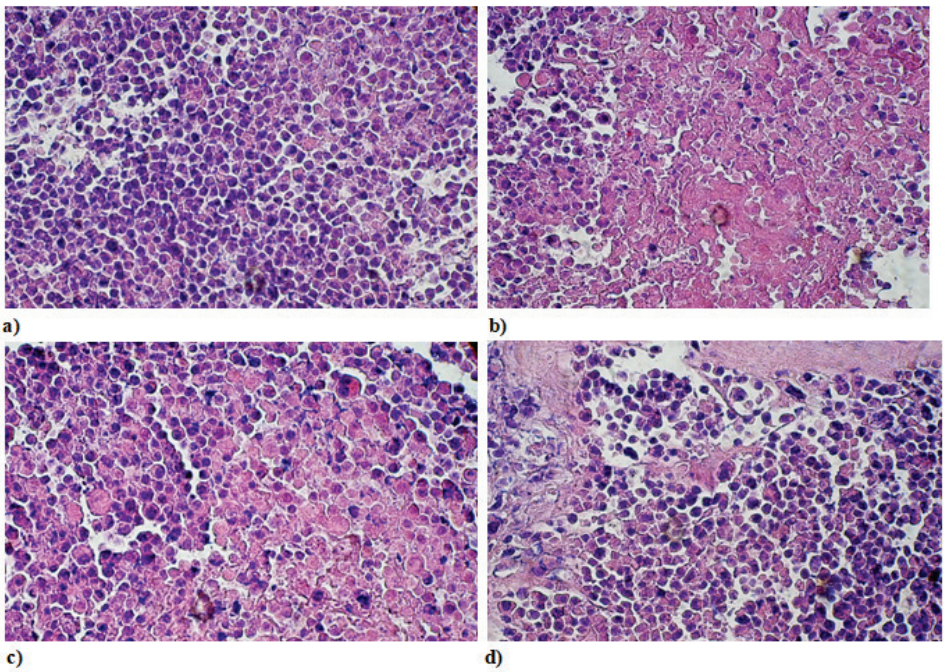

Fig. 3. Morphological picture of tumor tissue samples of Ehrlich carcinoma-bearing mice (H&E stained, x100)
a) Control – Hyperchromic atypical, polymorph nuclear cells;
b) M II-12 – Hyperchromic atypical cells on the periphery, significant tumor necrosis. Necrotic areas show dead tumor cells and inflammatory infiltrations;
c) Gz 9-10 – Hyperchromic polymorph nuclear atypical, cells. Areas of tumor necrosis;
d) NL 50 – Hyperchromic polymorph nuclear atypical, cells. Small areas of tumor necrosis in central areas.

This study aimed to assess the potential anti-cancer effects of the biologically active substances isolated from intracellular biomass/lysate obtained through solid-phase fermentation of microscopic fungi against Ehrlich solid tumor (EST) in mice.

The experimental findings presented in this study demonstrate the significant impact of biologically active substances, isolated from microscopic fungi on tumor growth and apoptotic processes in mice. The most substantial elevation was observed in mice treated with MII-12, where the caspase-3 level was increased by 0.58 ng per milliliter compared to the control group. This upregulation of caspase-3 indicates the induction of programmed cell death, aligning with the observed reduction in tumor volume.

The administration of MII-12 notably reduced tumor volume. It was remarkably decreased by 41% ($p<0.01$) compared to the control group. In contrast, NL 50 and Gz 9-10 did not reveal antitumor effects. These observations highlight the potent anti-tumor properties of MII-12.

The morphological study further supported the biochemical analysis, revealing distinct traces of apoptosis and massive necrosis in the tumor tissue. This convergence of morphological and biochemical evidence strengthens the conclusion that the observed reduction in tumor volume is associated with the induction of apoptosis, particularly under the influence of MII-12.

Conclusion

In conclusion, studying biologically active substances from microscopic fungi and their ability to activate caspase-3 to induce apoptosis in cancer cells represents a promising avenue in cancer research and therapy. This research area offers hope for innovative and targeted treatments that may enhance the outcomes and quality of life for cancer patients in the future.

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ბიოქიმია

მიკროსკოპული სოკოების გავლენა სიმსივნის ზრდაზე

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აპოპტოზი წარმოადგენს უმნიშვნელოვანეს ბიოლოგიურ პროცესს, რომელიც არეგულირებს უჯრედების სიცოცხლესა და სიკვდილს, გადამწყვეტ როლს ასრულებს ქსოვილების ჰომეოსტაზის შენარჩუნებაში. აპოპტოზის დისრეგულაციამ შესაძლოა გამოიწვიოს უჯრედების უკონტროლო პროლიფერაცია და სიმსივნის ზრდა. წარმოდგენილ ნაშრომში შესწავლილია მიკროსკოპული სოკოებიდან მიღებული ბიოლოგიურად აქტიური ნივთიერებების: MII-12, NL-50 და Gz-9-10 ეფექტი სიმსივნის ზრდასა და აპოპტოზურ პროცესებზე ერლიხის კარცინომის მატარებელ თაგვებში. კვლევის შედეგებმა აჩვენა, რომ MII-12-ს აქვს სიმსივნის საწინააღმდეგო ეფექტი. სიმსივნის ზრდა მნიშვნელოვნად შეფერხებული იყო საკონტროლო, NL-50 და Gz-9-10 სინჯით ნამკურნალებ ცხოველებთან შედარებით. მორფოლოგიურმა და ბიოქიმიურმა გამოკვლევებმა დაადასტურა MII-12 ბიოპრეპარატის სიმსივნის საწინააღმდეგო თვისებები. კასპაზა-3-ის დონე საგრძნობლად გაიზარდა, რაც მიუთითებს აპოპტოზის სტიმულაციაზე. სიმსივნური ქსოვილის მორფოლოგიურმა სურათმა გამოავლინა ნეკროზის ფართო უბნები. შეიძლება ითქვას, რომ სოკოვანი წარმოშობის ბიოლოგიურად აქტიური ნივთიერებები პერსპექტიულია სიმსივნის მკურნალობის ინოვაციური და მიზანმიმართული მეთოდების შემუშავებისთვის.

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