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Original Research Article

Surface Polyethylene Glycol Nanoliposome-encapsulated microRNA-495-3p Regulates mTOR Pathway and Improves Immune Cell Function in Colon Cancer Through Targeting GXYLT1

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Abstract

Background: miR-495-3p expression is related to tumor pathogenesis.
Objectives: This study explores the mechanism of the protective role of miR-495-3p in colon cancer and its interaction with glucoside xylosyltransferase 1 (GXYLT1) and mammalian target of rapamycin (mTOR) pathway.
Materials and Methods: A rat model of colon cancer was treated with polyethylene glycol (PEG) nanoliposomes, miR-495-3p, miR-495-3p-loaded PEG nanoliposomes, mTOR agonist, and mTOR inhibitor. After 1 week of intervention, rat colon tissues were taken for hematoxylin and eosin (HE) staining to identify the function of miR-495-3p-loaded PEG nanoliposomes on inflammation and gene expression was detected.
Results: miR-495-3p-loaded PEG nanoliposomes significantly inhibited cell proliferation and migration and had the lowest degree of infiltration and mitosis ($p < .05$). The addition of mTOR inhibitors further amplified the effect of miR-495-3p-loaded PEG nanoliposomes. Compared with miR-495-3p-loaded PEG nanoliposomes group, the GXYLT1 knockout + miR-495-3p-loaded PEG nanoliposomes had higher mTOR expression, and the addition of mTOR inhibitors decreased the level of mTOR ($p < .05$).
Conclusion: Encapsulation of miR-495-3p in PEG nanoliposomes can help enhance its targeting effect on colon cancer and improve the function of immune cells by inhibiting the level of GXYLT1 through regulation of the mTOR pathway. These findings provide a novel insight into nanoparticle-based gene therapy of colon cancer.

Keywords

Colon cancer, miR-495-3p, glucoside xylosyltransferase 1, mammalian target of rapamycin pathway, polyethylene glycol nanoliposomes

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Introduction

Colon cancer is a common digestive malignancy worldwide, with high morbidity and mortality. In China, it is the fourth most commonly diagnosed cancer (Bakr et al., 2023), responsible for 600,000 deaths every year (Eskelinen et al., 2023). Colon cancer is a serious threat to people's health and safety. Early detection and early treatment are the keys to improving the survival rate of patients. Currently, targeted therapy for colon cancer has become a research hotspot. MicroRNAs (miRNAs) are involved in tumorigenesis, where some miRNAs influence various regulatory pathways and affect the proliferation and apoptosis of tumor cells. Based on miRNAs and their possible regulated gene pairs, tumor treatment has

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become a current research hotspot (Guo et al., 2023). miR-495-3p is a newly discovered suppressor, and it is significantly downregulated in cervical and gastric cancer. Recent studies have shown that miR-495-3p plays a tumor suppressor role in various tumors. Alves and Geraldo (2023) found in their study that the overexpression of miR-495-3p could affect the expression of target genes such as TGFB2, EREG, and CCND1, thereby weakening the migration and invasion abilities of papillary thyroid cancer cells carrying BRAFV600E mutations; Geng et al. (2022) found through research that when the transcription of miR-495-3p was inhibited, its downstream KPNA2 was regulated, and the viability of melanoma cells was significantly enhanced; However, the mechanism of miR-495-3p in colon cancer has not been fully clarified.

Based on the potential of miRNAs in tumor treatment, how to improve their delivery efficiency has also become a key issue. Nanomaterials have been applied to clinical practice, and the materials carrying drugs and target genes carry drugs to tumor cells (Chi et al., 2020). Polyethylene glycol (PEG) liposome nanoparticles, characterized by low immunogenicity and good degradation, can increase the stability of loaded drugs or target genes. Combined with hydrophilic and lipophilic drugs, they are often used for drug loading, system construction, deoxyribonucleic acid (DNA) extraction, and so on. Encapsulation of drugs and target genes in PEG nanoliposomes can be mediated by receptors, which have good application value and research prospects in new nano-targeting systems. Based on this, in this study, we used PEG nanoliposomes to encapsulate miR-495-3p to enhance its targeting and bioavailability.

In recent years, accumulating evidence has shown that the mammalian target of rapamycin (mTOR) signaling pathway dysfunction is closely related to tumor formation, and mTOR can affect the immune function of the body and the progression of tumors (Bai et al., 2022; Cheng et al., 2023). Ai et al. (2018) pointed out that exosomes from tumor-associated fibroblasts activate the PI3K/AKT/mTOR signaling pathway, promoting the metastasis and growth of lung cancer cells. mTOR protein is highly expressed in colon cancer (Li et al., 2023), which suggests the importance of abnormal mTOR signaling to the pathogenesis of colon cancer. Zhang et al. (2021) furthermore noted that metformin can promote the proliferation and inhibit apoptosis of colon cancer cells through inhibition of the mTOR signaling pathway. Raja et al. (2023) showed that tumor-associated fibroblast exosomes, activating the mTOR signaling pathway, promote lung cancer cell growth. Previous studies have shown that glucoside xylosyltransferase 1 (GXYLT1) is also abnormally expressed in colon cancer (Peng et al., 2022). It can be used as one of the risk factors for colon cancer. However, the mechanism by which miR-495-3p targets GXYLT1 to regulate the mTOR pathway remains elusive. Can miR-495-3p affect the function of immune cells by targeting GXYLT1 to regulate the mTOR pathway in colon cancer? These questions deserve further investigation. Therefore, this study aims to establish a rat model of colon cancer to identify the effect of miR-495-3p-loaded PEG nanoliposomes on the mTOR pathway in colon cancer. This evidence might underlie the treatment of colon cancer and provide a reference for the clinical treatment of colon cancer by miR-495-3p.

Materials and Methods

Experimental Materials

A flow cytometry and enzyme-linked immunosorbent assay (ELISA) kits were obtained from the Third Military Medical University, primary antibodies from Abcam and secondary antibodies from Santa Cruz. Other reagents included CCK-8 kit (Beijing Biolab Technology Co., Ltd.); mTOR agonist, mTOR inhibitor (SANYO, Japan), and MTT (Wuhan Procell Biotechnology Co., Ltd.).

Male Sprague–Dawley (SD) rats were purchased from Jinan Jinfeng Experimental Animal Co., Ltd. The experiment was approved by the Animal Ethics Committee (Approval Number: GS20250168).

Experimental Methods

Construction of Colon Cancer Rat Model

A total of 100 SD rats (310 ± 10) g were raised in an environment of 22°C–28°C under a light/dark cycle of 12 h with free access to food and water. After 1 week of adaptive feeding, the DMH solution (US Sigma Company, TB1685, 25 mg/kg) was diluted to 0.5% with 0.9% NaCl, the pH was adjusted to 7.4, and then filtered and sterilized. It was subcutaneously injected once a week for 12 weeks. Through micro-ultrasound examination, a solid tumor with a diameter of ≥5 mm locally in the colon is regarded as successful modeling (for subsequent experiments), and if the rat dies during the modeling process, it is considered a failure.

Rat Grouping and Intervention

Eighty modeled rats were divided into eight groups: model group, PEG nanoliposome group, miR-495-3p group, miR-495-3p-loaded nanoliposome group, mTOR agonist group, mTOR inhibitor group, mTOR agonist + miR-495-3p-loaded nanoliposome group, mTOR inhibitor + miR-495-3p-loaded nanoliposome group (n = 10, each group). The rats received abdominal subcutaneous injection of 70 μg/kg PEG nanoliposome suspension, miR-495-3p-loaded PEG nanoliposomes suspension, or miR-495-3p suspension, and/or gavage of 2 mg/kg mTOR inhibitor or mTOR agonist, respectively. In addition, 20 successfully modeled rats were knocked out of the GXYLT1 gene (the knockout of the GXYLT1 gene is achieved through CRISPR/Cas9 technology, and the targeted sequence is 5′-AGCTTTAAAGGCAGACTTGAC-3′) and

randomly divided into two groups. One group received an abdominal subcutaneous injection of 70 μg/kg miR-495-3p-loaded nanoliposome, and the other group further received additional intragastric administration of mTOR inhibitor (2 mg/kg) once a day for 1 week. During the group intervention process, rats were grouped according to the random number table method, and the experimenter blinded the group information to avoid operational bias.

Sample Size Calculation

Based on the pre-experiment effect size ($\alpha = 0.05$, $\beta = 0.2$), using the general statistical standard ($\alpha = 0.05$, power = 80%), after analysis with G*Power software, it was assumed that there were at least eight rats in each group. Considering the "3R principle" of animal experiments, to prevent animal death or experimental failure, it was expanded to 10 rats per group.

Pathological Tissue Sampling

Colonic tissue and blood collection: After treatment, the blood of rats in each group was collected and stored for later use. The rats in each group were fasted for 24 h. Under anesthesia with 2% sodium pentobarbital, the rats were sacrificed, and approximately 1 cm of colon tissue containing the tumor was taken and fixed with 4% paraformaldehyde.

Cell Collection

Cells of the colon tissues were suspended to obtain a single-cell suspension (2×10^6 cells/mL) for future use.

Hematoxylin and Eosin (HE) Staining

The removed colon tissue was dehydrated, embedded, and sliced. After the prepared slices were rinsed thoroughly with NS, they were effectively fixed with formalin and rinsed with sterile distilled water again after 30 min. The slices were stained with hematoxylin and $NaHCO_3$ solution after 15 min. The sample was then stained with eosin solution again, rinsed with sterile distilled water again, cleared with acetone-xylene (2 min/time, 3 times) and pure xylene (5 min), and sealed in gum solution, and then observed under a microscope.

MTT Assay

Cells were inoculated and cultured for 2 days, and then immersed in 20 μL of MTT solution for 4 h. With the supernatant discarded, dimethyl sulfone solution was added, and finally, the density value was detected.

Flow Cytometry

Cells were digested with trypsin and transferred to the EP tube. Following centrifugation at 1,000 r/min for 5 min, the sample was washed with phosphate-buffered saline (PBS), 5 min × 3 times, and fixed with 75% ethanol overnight, incubated in RNase solution. Half an hour later, the cells were placed in PI staining solution, cultured in the dark, and detected by a FACSCalibur flow cytometer.

Transwell Detection

The upper chamber was coated with Matrigel gel (culture medium:gel: 1:5) dried. The cells were inoculated at a density of 3×10^5 cells/mL in the upper chamber, and serum culture solution was added to the lower chamber, cultivated for 24 h. The sample was fixed with formaldehyde, permeabilized with methanol, dried, and photographed.

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from rat colon tissue cells by the TRIzol method, and was reversely transcribed into complementary DNA (cDNA). RT-qPCR was performed using SYBR fluorescent PCR technology. With β-actin as an internal reference, the gene expressions were estimated using the $2^{-\Delta\Delta Ct}$ method and the Step One Plus real-time PCR system. The primer sequences are shown in Table 1.

Western Blot

Cells were inoculated, observed, and the protein expression was detected when the cells adhered to about 90%. The cells were incubated with lysate and centrifuged. The upper layer of lysate was collected, followed by protein quantification. The protein sample was separated and then transferred to the membrane and probed with the primary antibodies (1:1: 5,000) and the secondary antibodies (1:1: 5,000). Then, the

Table 1. Primer Sequences for Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR).

Genes		Sequence
mTOR	Forward	5′-GACCGTCTGAGTGGGAAG-3′
	Reverse	5′-TTACCAGAAAGCGCACCAG-3′
GXYLT1	Forward	5′-AGCTTTAAAG-GCAGACTTGAC-3′
	Reverse	5′-CT-CACTTGGAAAGGTTATGGG-3′
β-actin	Forward	5′-TTCCACCCTTCCCTG-3′
	Reverse	5′-CGGACTCGTCATACTCCTCCT-3′

Note: GXYLT1: Glucoside xylosyltransferase 1; mTOR: Mammalian target of rapamycin.

blot was washed, exposed, developed, and imaged with the exposure time adjusted to determine the best band.

ELISA Method

The rat blood was centrifuged to obtain the upper serum, and the serum levels of interleukin (IL)-2 and IL-10 in each group were measured strictly in accordance with the ELISA kit instructions, and the optical density (OD) value was detected.

Statistical Analysis

Data were analyzed by SPSS 21.0 and GraphPad Prism, and the calculated data conforming to normal distribution and homogeneity of variance were expressed as (mean ± SD); all data calculations were performed using the *F*-test, and the comparison between groups was performed using the least significant difference (LSD) method. $p < .05$ indicates a significant difference.

Results

miR-495-3p-loaded PEG Nanoliposomes Inhibit Proliferation and Migration of Colon Cancer Cells and Improve the Function of Immune Cells

Among the 100 rats, 97 survived until the end of the experiment (with a survival rate of 97%), and 3 died due to injection complications. After administration of miR-495-3p, the cell proliferation inhibition rate, apoptosis rate and serum IL-10 levels were significantly increased (Figure 1A,B,F), while the amount of migrated and invaded cells and serum IL-2 levels were significantly decreased (Figure 1C–1E, $p < .05$). This evidence confirms the impact of miR-495-3p on colon cancer cell growth. Furthermore, it was found that miR-495-3p-loaded PEG nanoliposomes promoted colon cancer cell apoptosis, suppressed malignant characteristics of cells, and improved immune cell function more significantly (Figure 1A–1F), which demonstrates that the targeting effect of miR-495-3p-loaded PEG nanoliposomes is more significant and has great biological efficacy.

Further HE staining results showed that lymphocyte infiltration and nuclear division in the miR-495-3p group were already alleviated (vs. the model group), and the presence of miR-495-3p-loaded PEG nanoliposomes further relieved the symptoms (Figure 1G, $p < .05$). The results of the cell invasion experiment can be seen (Figure 1H). Compared with the model group, the number of transmembrane cells in the miR-495-3p group decreased by 42.3%, while the inhibitory effect of the miR-495-3p-loaded PEG nanoliposomes group was more significant. Meanwhile, compared with the model group, treatment with miR-495-3p alone reduced the number of migrating cells, while the inhibition rate increased after encapsulation with nano-liposomes (Figure 1I). These further confirm that miR-495-3p-loaded PEG nanoliposomes have a significant effect on inhibiting colon cancer and improving immune cell function.

miR-495-3p-loaded PEG Nanoliposomes Inhibit Colon Cancer Through Regulating the Expression of mTOR

As revealed by RT-qPCR, the mTOR level of the miR-495-3p group was lower than that of the model group, whilst the lowest level was detected in the PEG miR-495-3p-loaded PEG nanoliposome group (Figure 2A), suggesting the function of miR-495-3p in the modeled rats might be associated with reduced mTOR levels. In order to verify this speculation, mTOR agonists were applied to the rats under the intervention of miR-495-3p-loaded PEG nanoliposome, and the mTOR level was found to be increased, but lower than the model group and mTOR agonist group (Figure 2B, $p < .001$). Additional treatment with mTOR inhibitors even further amplified the effect of the PEG nanoliposomes ($p < .001$), which fully explained the reduced expression of mTOR, which might be responsible for the effect of miR-495-3p-loaded PEG nanoliposomes on colon cancer.

PEG Nano-lipid Encapsulation of miR-495-3p Inhibits the Expression of GXYLT1 and Inhibits the level of mTOR

The experimental results have noted that the impact of miR-495-3p-loaded PEG nanoliposomes on colon cancer rats is produced by inhibiting mTOR levels. The relative content of GXYLT1 in rats was checked. XYLT1 expression was dramatically decreased in the loaded-PEG nanoliposomes group relative to the model group (Figure 3A and 3B, $p < .05$), suggesting that PEG nano-lipid encapsulation of miR-495-3p might suppress the expression of GXYLT1. To elucidate the interaction between miR-495-3p and GXYLT1, GXYLT1 knockout rats were selected as the research object. Interestingly, after silencing GXYLT1 based on miR-495-3p-loaded PEG nanoliposomes, the expression level of mTOR increased (vs. miR-495-3p-loaded PEG nanoliposomes group). The addition of mTOR inhibitor decreased the mTOR level, which, however, was still higher than that of the miR-495-3p-loaded PEG nanoliposome group (Figure 3C). The above results proved that after the miR-495-3p intervention, GXYLT1 expression declined, and the mTOR level also decreased further.

Discussion

At present, studies on the pathogenesis of colon cancer have unveiled the roles of some miRNAs in the tumorigenesis of colon cancer. miRNAs can positively and negatively affect target genes, thereby regulating intracellular signaling pathways,

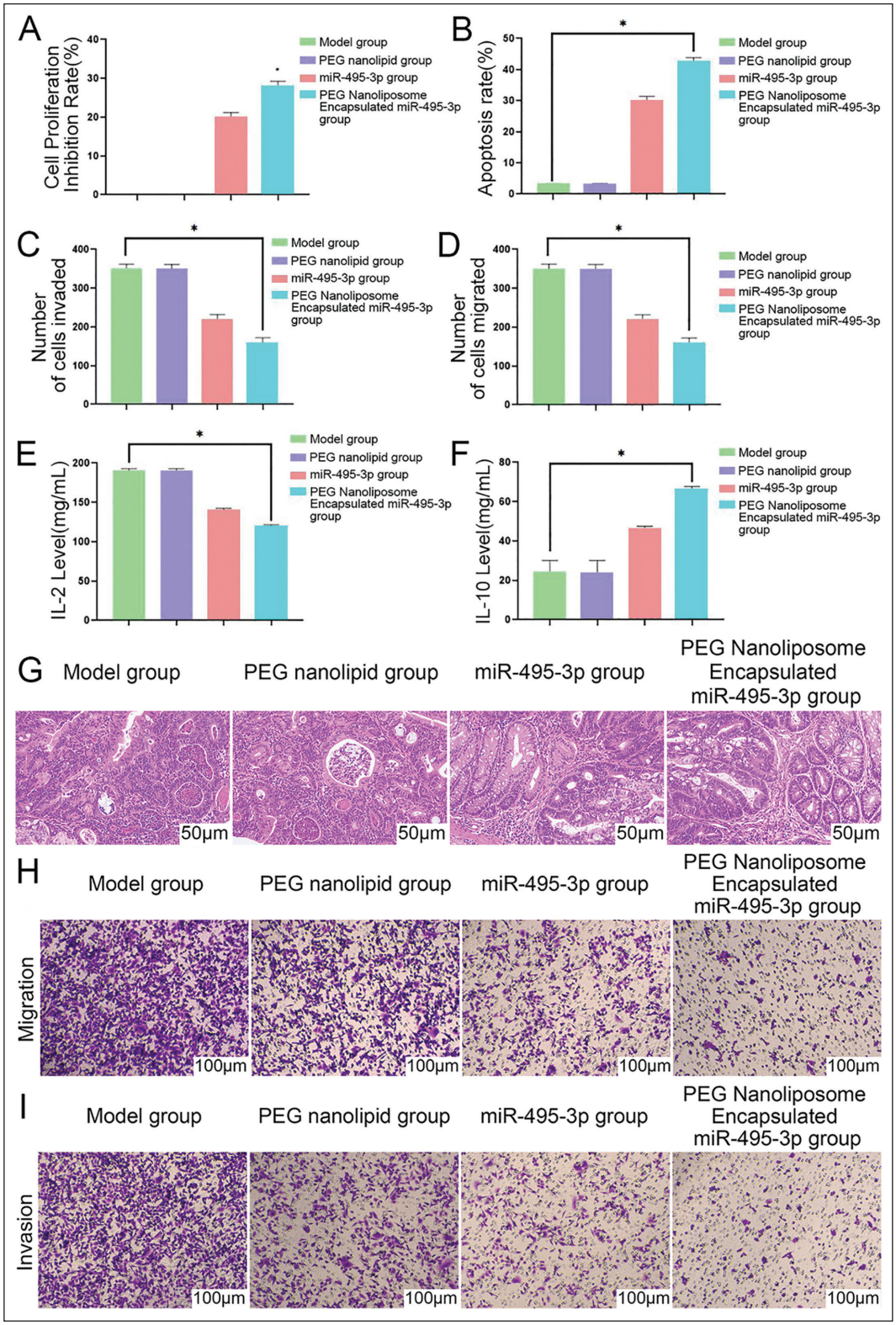


Figure 1. Analysis of Colon Cancer Cell Proliferation, Apoptosis, Migration, Invasion, and Immune Factor Levels (n = 5). (A) Quantification Diagram of Cell Proliferation Inhibition Rate Detected by MTT assay; (B) Quantification Diagram of Cell Apoptosis Detected by Flow Cytometry; (C, D) Quantification Diagram of Cell Invasion and Migration Detected by Transwell; (E, F) Interleukin (IL)-2 Detected by Enzyme-linked Immunosorbent Assay (ELISA) Method, Quantification Diagram of IL-10 Factor Level; (G) Histopathological Morphology Diagram of Rat Colon Under Hematoxylin and Eosin (HE) Staining; (H) Cell Invasion Diagram Detected by Transwell; (I) Cell Migration Diagram Detected by Transwell; Compared with the Model Group, *p < .05.

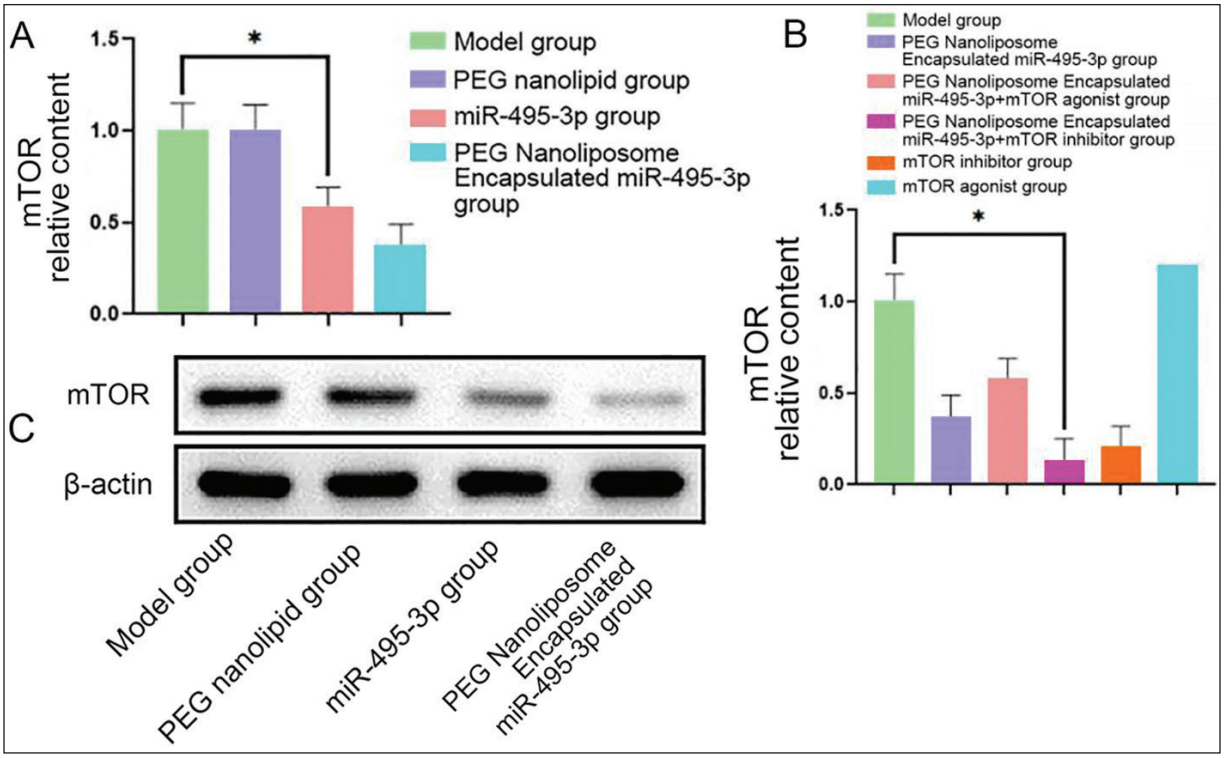


Figure 2. Relative Content of Mammalian Target of Rapamycin (mTOR) (n = 5). (A, B) Quantification of mTOR Expression Detected by Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR); (C) mTOR Expression Detected by Western Blot; Compared with the Model Group, *p < .05.

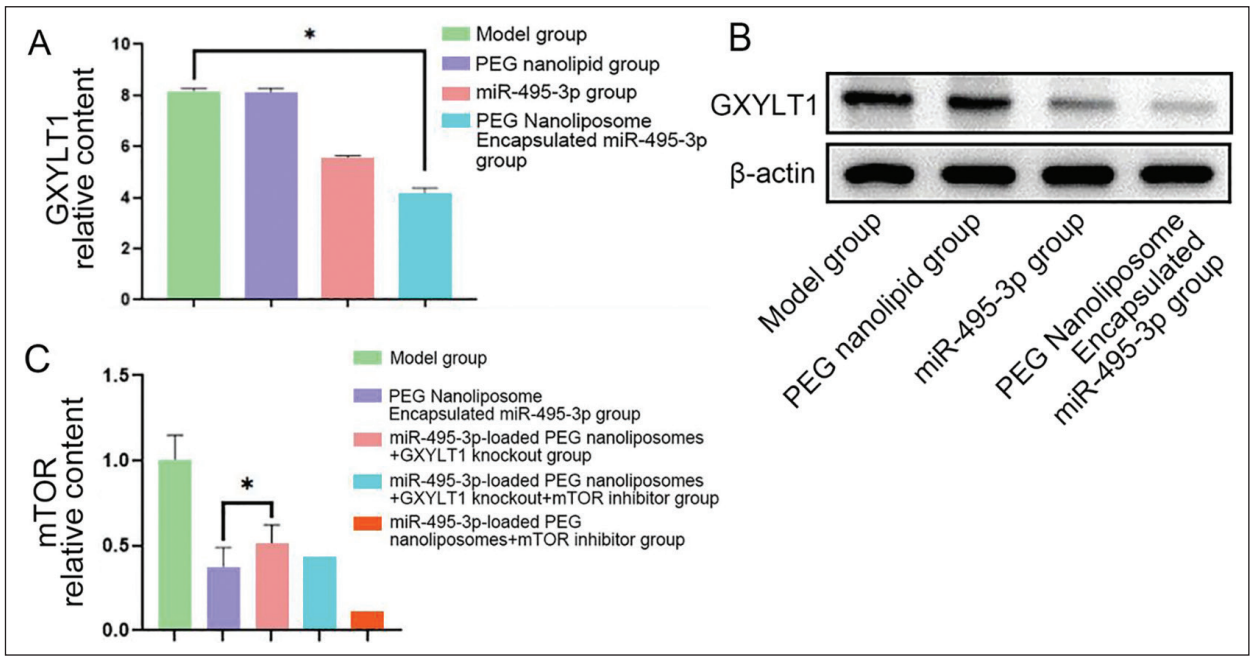


Figure 3. Glucoside Xylosyltransferase 1 (GXYLT1) and Mammalian Target of Rapamycin (mTOR) Content (n = 5). (A) Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis of GXYLT1 Expression; (B) Western Blot to Detect GXYLT1 Expression; (C) RT-qPCR Analysis of mTOR Expression and Quantification; Compared with the Model Group, *p < .05.

and then cell metabolism, proliferation and apoptosis. miR-495-3p is reported to target and regulate GXYLT1, and inhibit the progression of colon cancer (Peng et al., 2022).

Chen et al. (2020) found through animal experiments that miR-495-3p can mediate the expression of IL-6, hindering the metastasis and proliferation of cervical cancer cells. Chen et al. (2022) also noted miR-495-3p regulates the Wnt signaling pathway, as miR-495-3p treatment suppresses the migration of retinoblastoma cells and induces apoptosis activity. It can be seen that miR-495-3p plays an important role in the occurrence and development of early tumors. In recent years, an increasing number of studies have found that the expression level of miR-495-3p has a certain relationship with the biological activity of colon cancer cells (Fan et al., 2023; Gasparello et al., 2022). Research by Guo and Sun (2022) showed that miR-495 can mediate FAM83D expression, thereby inhibiting cell migration and proliferation in the body. Besides, Huang et al. (2022) also showed that, compared with normal colon tissue, the expression level of miR-495-3p in colon cancer was low, and the cycle progression of colon cancer cells could be inhibited by up-regulating miR-495-3p. In this study, a rat model of colon cancer was prepared, and HE staining was performed on the colon tissue. The tissue staining results showed that treatment with miR-495-3p alleviated lymphocyte infiltration and nuclear division in rat colon tissue and hindered the process of cancer cell growth, including proliferation and migration. This indicates that miR-495-3p has a certain inhibitory effect on colon cancer. The treatment technology of wrapping drugs or target genes with nanomaterials has become a hot spot in clinical research, especially in anti-tumor aspects. In this study, PEG nano-lipid particles were successfully prepared to encapsulate miR-495-3p. After intervention in rats, the results showed that miR-495-3p-loaded PEG nanoliposomes exhibited a strong anti-tumor effect. Most notably, analysis suggests that miR-495-3p can be efficiently transported into cells after being encapsulated by PEG nano-lipid particles, enhancing its targeting effect and thereby improving the therapeutic effect. Hu et al. (2020) showed that PEG-modified $NaHCO_3$ lipid microbubbles can improve the antigen presentation efficiency of dendritic cells. Enlarging the exposure area of tumor antigens, the nanoparticles might strengthen the anti-tumor effect of drugs.

Studies have pointed out that mTOR can not only affect the synthesis and secretion of proteins in the body, but also affect the proliferation of tumor cells (Lutz et al., 2023). Angiogenesis is an essential part of tumor cell proliferation and migration. Inhibiting mTOR expression can effectively delay tumor angiogenesis (Ma et al., 2023). The research results of Qin et al. (2023) show that the mTOR signaling pathway is highly expressed in pituitary gonadotroph adenomas, and drugs inhibiting the mTOR signaling pathway can effectively inhibit cell proliferation. In the animal experiments of Sidorov et al. (2023), the expression of p-mTOR in the tumors of lung cancer mice in the anti-cancer drug group was decreased. To identify the mechanism of miR-495-3p inhibiting colon cancer, we checked the expression level of mTOR, and found that the mTOR level was lower in rats in the miR-495-3p group. The reason for the analysis was that miR-495-3p decreases mTOR expression, as mTOR is highly expressed in tumors. This indicates that inhibiting the expression of mTOR can effectively suppress tumor activity. Tan et al. (2023) revealed that the antagonistic effect of Jianpi Xiaoai formula on the metastasis and recurrence of colon cancer may be associated with its inhibition of the Akt/mTOR signaling pathway. Trpkov (2023) also pointed out that the apoptosis of human colon cancer HCT116 cells may be related to the inhibition of the mTOR signaling pathway. The above studies all show that inhibiting the mTOR signaling pathway in the body can inhibit colon cancer.

miR-495-3p might affect the autophagy of tumor cells by inhibiting mTOR (Wang et al., 2023; Yilmaz et al., 2023). To confirm the inhibitory role of miR-495-3p, mTOR inhibitors were administered on the basis of miR-495-3p treatment. Compared with miR-495-3p alone, the addition of the mTOR inhibitor decreased mTOR levels, while after using the mTOR agonists, mTOR levels increased (vs. the miR-495-3p group, the miR-495-3p+mTOR inhibitor group), which further confirmed that miR-495-3p inhibited the mTOR effect. mTOR has been highlighted as a central regulatory factor of immune response, regulating immune cells like T cells and natural killer (NK) cells. Studies have confirmed that mTOR can promote the production of IL-2 and inhibit IL-10 expression by regulating immune cells (Alamri et al., 2024; Chen et al., 2023). In this study, IL-2 and IL-10 levels were checked. After the intervention of miR-495-3p, IL-2 in rat serum decreased, and IL-10 increased. Therefore, it can be concluded that miR-495-3p plays an anti-tumor role by inhibiting mTOR and strengthening the function of immune cells. Additionally, the study demonstrated that miR-495-3p also inhibited GXYLT1 expression. Consistently, the research results of Liu et al. (2023) confirmed that miR-495-3p promotes apoptosis and suppresses proliferation by reducing GXYLT1. In this study, the expression of GXYLT1 in rats after miR-495-3p intervention decreased. In addition, the research by Yang, Zhang et al. (2023) also showed that miR-495-3p regulates GXYLT1 to regulate periodontal inflammation and decrease the expression and phosphorylation of mTOR. Therefore, to further clarify the mechanism of miR-495-3p targeting GXYLT1 to regulate mTOR in colon cancer, further experiments were conducted in this study. Compared with only miR-495-3p intervention rats, the mTOR level of rats in the GXYLT1-knockout group was higher, while the mTOR level decreased after the application of the mTOR inhibitor, indicating that miR-495-3p can reduce the expression of GXYLT1 and the level of mTOR. The analysis of the possible reason is that GXYLT1 knockout activates the upstream PI3K signaling pathway through a feedback mechanism, resulting in a temporary increase in mTOR (Yang, Yu, et al., 2023); however, after combining with mTOR inhibitors, this pathway was blocked, and the mTOR level dropped. However, this specific mechanism still needs further exploration.

Conclusion

Collectively, miR-495-3p-loaded PEG nanoliposomes can effectively inhibit the expression of GXYLT1 and mTOR to suppress colon cancer cell growth and improve cellular immune function. This finding breaks through the bottleneck of clinical colon cancer treatment. However, this study also has some limitations. For instance, the relationship between immune cell function and mTOR signaling pathway and the mechanism of action needs to be further clarified. In addition, how miR-495-3p mediates GXYLT1 and participates in the progression of colon cancer remains elusive. The pathogenesis of the disorder is relatively complex. Whether miR-495-3p may also regulate the mTOR signaling pathway by regulating other genes deserves further investigation. Furthermore, this study only observed short-term therapeutic effects in animals and requires long-term experimental verification. There is also a lack of *in vitro* cell experiments to support it. Meanwhile, clinical transformation still requires further evaluation of human safety and efficacy.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study was approved by the ethics committee of the School of Clinical Medicine, Jiangxi Medical College.

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Informed Consent

Not applicable.

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References

Ai, X., Mao, F., Shen, S., Shentu, Y., Wang, J., & Lu, S. (2018). Bexarotene inhibits the viability of non-small cell lung cancer cells via slc10a2/PPARgamma/PTEN/mTOR signaling pathway. *BMC Cancer*, *18*(1), 407. https://doi.org/10.1186/s12885-018-4224-x

Alamri, A. H., Debnath, S., Alqahtani, T., Alqahtani, A., Alshehri, S. A., & Ghosh, A. (2024). Enhancing plant-derived smart nano inhibitor in targeting mammalian target of rapamycin (mTOR) in breast cancer using *Curcuma longa*-derived compound curcumin. *Environmental Science and Pollution Research International*, *31*(34), 46462–46469. https://doi.org/10.1007/s11356-023-25375-0

Alves, L. F., & Geraldo, M. V. (2023). MiR-495-3p regulates cell migration and invasion in papillary thyroid carcinoma. *Frontiers in Oncology*, *13*, Article 1039654. https://doi.org/10.3389/fonc.2023.1039654

Bai, S., Wu, Q., Zhu, S., Zhang, Y., Chen, X., Su, M., Pan, J., Li, S., Yue, T., Xu, L., Xie, D., Tian, C., Zhao, D., Li, X., Hou, J., Wang, L., Fu, S., Xue, Y., Jiang, A., … Bai, L. (2022). Vam6 reduces iNKT cell function in tumor via modulating AMPK/mTOR pathways. *Frontiers in Immunology*, *13*, Article 1051045. https://doi.org/10.3389/fimmu.2022.1051045

Bakr, M., Abd-Elmawla, M. A., Elimam, H., Gamal El-Din, H., Fawzy, A., Abulsoud, A. I., & Rizk, S. M. (2023). Telomerase RNA component lncRNA as potential diagnostic biomarker promotes CRC cellular migration and apoptosis evasion via modulation of beta-catenin protein level. *Non-Coding RNA Research*, *8*(3), 302–314. https://doi.org/10.1016/j.ncrna.2023.03.004

Chen, B., Zheng, D., Liu, C., Bhandari, A., Hirachan, S., Shen, C., Mainali, S., Li, H., Jiang, W., Xu, J., Zhang, X., Tang, K., & Zhang, W. (2023). S100A6 promotes the development of thyroid cancer and inhibits apoptosis of thyroid cancer cells through the PI3K/AKT/mTOR pathway. *Pathology, Research and Practice*, *242*, Article 154325. https://doi.org/10.1016/j.prp.2023.154325

Chen, D., Qin, Y., Dai, M., Li, L., Liu, H., Zhou, Y., Qiu, C., Chen, Y., & Jiang, Y. (2020). BGN and COL11A1 regulatory network analysis in colorectal cancer (CRC) reveals that BGN influences CRC cell biological functions and interacts with miR-6828-5p. *Cancer Management and Research*, *12*, 13051–13069. https://doi.org/10.2147/CMAR.S277261

Chen, X., Xu, M., Xu, X., Zeng, K., Liu, X., Sun, L., Pan, B., He, B., Pan, Y., Sun, H., Xia, X., & Wang, S. (2022). Retraction notice to: METTL14 suppresses CRC progression via regulating N6-methyladenosine-dependent primary miR-375 processing. *Molecular Therapy*, *30*(7), 2640. https://doi.org/10.1016/j.ymthe.2022.03.017

Cheng, T. D., Zhang, R., Gong, Z., Qin, B., Cannioto, R. A., Datta, S., Zhang, W., Omilian, A. R., Yao, S., Khoury, T., Hong, C.-C., Bandera, E. V., & Ambrosone, C. B. (2023). Association between recreational physical activity and mTOR signaling pathway protein expression in breast tumor tissue. *Cancer Research Communications*, *3*(3), 395–403. https://doi.org/10.1158/2767-9764.CRC-22-0405

Chi, L., Xu, C., Li, S., Wang, X., Tang, D., & Xue, F. (2020). Thionine-doped nanometer-sized silica conjugated with phenylboronic acid: An innovative recognition/signal element for voltammetric aptasensing of colorectal cancer-related carcinoembryonic antigen. *Analytica Chimica Acta*, *1136*, 91–98. https://doi.org/10.1016/j.aca.2020.08.029

Eskelinen, M., Meklin, J., Guimaraes, D. P., Selander, T., Syrjänen, K., & Eskelinen, M. (2023). Diagnostic accuracy of fecal immunochemical test (FIT) in bleed-positive and bleed-negative colorectal cancer (CRC) among a cohort of 5,090 subjects who participated in the colorectal neoplasia (CRN) screening in Brazil. *Anticancer Research*, *43*(4), 1569–1580. https://doi.org/10.21873/anticanres.16307

Fan, L., Li, W., & Jiang, H. (2023). Circ_0000395 promoted CRC progression via elevating MYH9 expression by sequestering miR-432-5p. *Biochemical Genetics*, *61*(1), 116–137. https://doi.org/10.1007/s10528-022-10245-0

Gasparello, J., Papi, C., Zurlo, M., Gambari, L., Manicardi, A., Rozzi, A., Ferrarini, M., Corradini, R., Gambari, R., & Finotti, A. (2022). MicroRNAs miR-584-5p and miR-425-3p are up-regulated in plasma of colorectal cancer (CRC) patients: Targeting with inhibitor peptide nucleic acids is associated

with induction of apoptosis in colon cancer cell lines. *Cancers*, *15*(1), Article 128. https://doi.org/10.3390/cancers15010128

Geng, X., Qiu, X., Gao, J., Gong, Z., Zhou, X., Liu, C., & Luo, H. (2022). CREB1 regulates KPNA2 by inhibiting mir-495-3p transcription to control melanoma progression: The role of the CREB1/miR-495-3p/KPNA2 axis in melanoma progression. *BMC Molecular and Cell Biology*, *23*(1), 57. https://doi.org/10.1186/s12860-022-00446-1

Guo, S., & Sun, Y. (2022). OTOP2, inversely modulated by miR-3148, inhibits CRC cell migration, proliferation and epithelial-mesenchymal transition: Evidence from bioinformatics data mining and experimental verification. *Cancer Management and Research*, *14*, 1371–1384. https://doi.org/10.2147/CMAR.S345299

Guo, X., Yang, F., Yu, L., Wen, R., Zhang, X., & Lin, H. (2023). MiR-598-5p inhibits breast cancer tumor growth and lung metastasis by targeting PPAPDC1A. *The Chinese Journal of Physiology*, *66*(2), 103–110. https://doi.org/10.4103/cjop.CJOP-D-22-00089

Hu, P., Shang, L., Chen, J., Chen, X., Chen, C., Hong, W., Huang, M., Xu, P., & Chen, Z. (2020). A nanometer-sized protease inhibitor for precise cancer diagnosis and treatment. *Journal of Materials Chemistry B*, *8*(3), 504–514. https://doi.org/10.1039/c9tb02081k

Huang, Q., Yang, Z., Tian, J.-H., You, P.-D., Wang, J., Ma, R., Yu, J., Zhang, X., Cao, J., Cao, J., & Wang, L.-B. (2022). LncSNHG1 promoted CRC proliferation through the miR-181b-5p/SMAD2 axis. *Journal of Oncology*, *2022*, Article 4181730. https://doi.org/10.1155/2022/4181730

Li, J., Chen, L., Pang, J., Yang, C., Xie, W., Shen, G., Chen, H., Li, X., Xiao, S.-Y., & Li, Y. (2023). Autophagy-related gene WD repeat domain 45B promotes tumor proliferation and migration of hepatocellular carcinoma through the Akt/mTOR signaling pathway. *Diagnostics*, *13*(5), Article 906. https://doi.org/10.3390/diagnostics13050906

Liu, J., Wang, H., Xiao, S., Zhang, S., Qi, Y., & Wang, M. (2023). Circ_0000231 promotes paclitaxel resistance in ovarian cancer by regulating miR-140/RAP1B. *American Journal of Cancer Research*, *13*(3), 872–885.

Lutz, V., Hellmund, V. M., Picard, F. S. R., Raifer, H., Ruckenbrod, T., Klein, M., Bopp, T., Savai, R., Duewell, P., Keber, C. U., Weigert, A., Chung, H.-R., Buchholz, M., Menke, A., Gress, T. M., Huber, M., & Bauer, C. (2023). IL18 receptor signaling regulates tumor-reactive CD8+ T-cell exhaustion via activation of the IL2/STAT5/mTOR pathway in a pancreatic cancer model. *Cancer Immunology Research*, *11*(4), 421–434. https://doi.org/10.1158/2326-6066.CIR-22-0398

Ma, C., Wei, X., Wang, F., Zhang, T., Jiang, Y., Meng, Z., & Zhang, Z. (2023). Tumor necrosis factor alpha-induced protein 3 mediates inflammation and neuronal autophagy in Parkinson's disease via the NFkappaB and mTOR pathways. *Neuroscience Letters*, *805*, Article 137223. https://doi.org/10.1016/j.neulet.2023.137223

Peng, L., Zhao, M., Liu, T., Chen, J., Gao, P., Chen, L., Xing, P., Wang, Z., Di, J., Xu, Q., Qu, H., Jiang, B., & Su, X. (2022). A stop-gain mutation in GXYLT1 promotes metastasis of colorectal cancer via the MAPK pathway. *Cell Death and Disease*, *13*(4), 395. https://doi.org/10.1038/s41419-022-04844-3

Qin, S., Wang, G., Chen, L., Geng, H., Zheng, Y., Xia, C., Wu, S., Yao, J., & Deng, L. (2023). Pharmacological vitamin C inhibits mTOR signaling and tumor growth by degrading Rictor and inducing HMOX1 expression. *PLoS Genetics*, *19*(2), Article e1010629. https://doi.org/10.1371/journal.pgen.1010629

Raja, K., Vignesh, A., Lavanya, P., Ravi, M., Selvakumar, S., & Vasanth, K. (2023). Organosulfur compound identified from *Striga angustifolia* (D. Don) C.J. Saldanha inhibits lung cancer growth and induces apoptosis via p53/mTOR signaling pathway. *Applied Biochemistry and Biotechnology*, *195*(12), 7277–7297. https://doi.org/10.1007/s12010-023-04467-0

Sidorov, M., Dighe, P., Woo, R. W. L., Rodriguez-Brotons, A., Chen, M., Ice, R. J., Vaquero, E., Jian, D., Desprez, P.-Y., Nosrati, M., Galvez, L., Leng, L., Dickinson, L., Kashani-Sabet, M., McAllister, S. D., & Soroceanu, L. (2023). Dual targeting of EGFR and MTOR pathways inhibits glioblastoma growth by modulating the tumor microenvironment. *Cells*, *12*(4), Article 547. https://doi.org/10.3390/cells12040547

Tan, X., Zhang, Z., Yao, H., & Shen, L. (2023). Retraction notice to "Tim-4 promotes the growth of colorectal cancer by activating angiogenesis and recruiting tumor-associated macrophages via the PI3K/AKT/mTOR signaling pathway" [Canc. Lett. 436(2018), 119–128]. *Cancer Letters*, *558*, Article 216060. https://doi.org/10.1016/j.canlet.2023.216060

Trpkov, K. (2023). TSC/MTOR-associated eosinophilic renal tumors exhibit a heterogeneous clinicopathologic spectrum: Several distinct entities or a tumor family? *The American Journal of Surgical Pathology*, *47*(4), 518–519. https://doi.org/10.1097/PAS.0000000000002020

Wang, S., Shi, J.-T., Wang, X.-R., Mu, H.-X., Wang, X.-T., Xu, K.-Y., Wang, Q.-S., & Chen, S.-W. (2023). 1H-Indazoles derivatives targeting PI3K/AKT/mTOR pathway: Synthesis, anti-tumor effect and molecular mechanism. *Bioorganic Chemistry*, *133*, Article 106412. https://doi.org/10.1016/j.bioorg.2023.106412

Yang, F., Zhang, J., Zhao, Z., Liu, Y., Zhao, Z., Fu, K., Li, B., & Jin, J. (2023). Artemisinin suppresses aerobic glycolysis in thyroid cancer cells by downregulating HIF-1a, which is increased by the XIST/miR-93/HIF-1a pathway. *PLOS One*, *18*(4), Article e0284242. https://doi.org/10.1371/journal.pone.0284242

Yang, X., Yu, F., Huang, G., Ni, Y., Zhang, T., Zou, Z., & Meng, M. (2023). Exosomal miR-133a-3p promotes the growth and metastasis of lung cancer cells following incomplete microwave ablation. *International Journal of Hyperthermia*, *40*(1), Article 2190065. https://doi.org/10.1080/02656736.2023.2190065

Yılmaz, S., Doğanyiğit, Z., Oflamaz, A. O., Ateş, Ş., Söylemez, E. S. A., Nisari, M., & Farooqı, A. A. (2023). Determination of rutin's antitumoral effect on EAC solid tumor by AgNOR count and PI3K/AKT/mTOR signaling pathway. *Medical Oncology*, *40*(5), 131. https://doi.org/10.1007/s12032-023-01999-7

Zhang, Y., Zhao, Y., Guo, J., Cui, H., & Liu, S. (2021). Anticancer activity of safranal against colon carcinoma is due to induction of apoptosis and G2/M cell cycle arrest mediated by suppression of mTOR/PI3K/Akt pathway. *Journal of BUON*, *26*(1), 297.