ORIGINAL RESEARCH

Inhibition of Acetylcholine Expression in the Tumor Microenvironment by Mustard Oil: A Potential Strategy to Retard Colon Cancer Progression

Hu Chen, MD; Na Wang, MD; Shunli Wei, MD; Xinjian Xu, PhD; Chunxiao Wu, MD; Shugang Liu, MD

ABSTRACT

Objective • This study aims to investigate the potential of mustard oil-induced reduction in acetylcholine expression as a means to delay the progression of colon cancer within the internal environment.

Methods • The study design in this research involved both *in vitro* cellular experiments and *in vivo* animal experiments to employ mustard oil to modulate acetylcholine expression levels and evaluate its impact on colon cancer. Cellular experiments involved the introduction of six concentrations of acetylcholine (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} mol/L) into colon cancer cell cultures to monitor cell proliferation. Animal experiments encompassed the subcutaneous CT26 colon cancer cells implantation into 28 Balb/c mice, divided into experimental and control groups. After tumor establishment, both groups were fed standard diets for two weeks. Serum acetylcholine concentrations were measured from eye blood samples.

Additionally, Balb/c mice were inoculated with CT26-derived colon cancer cells and further categorized into experimental and control groups. A total of 14 mice comprised each group, with experimental mice fed mustard oil and control mice fed soybean oil. Post two weeks, serum acetylcholine expression in both groups was assessed. After sacrifice, subcutaneous tumors were excised, and tumor dimensions were measured using a Vernier scale.

Results • Acetylcholine concentration augmentation in the culture medium corresponded to gradual cell proliferation escalation, peaking at 10^{-5} mol/L, exhibiting statistical significance. Comparative analysis revealed significantly elevated relative acetylcholine expression levels in Balb/c mice with tumor-bearing colon cancers compared to normal Balb/c mice. Experimental group mice exhibited substantially lower serum acetylcholine concentrations than control group mice. Mustard oil administration effectively curtailed acetylcholine expression in normal Balb/c mice, consequently retarding tumor growth. These findings underscore mustard oil's potential to diminish serum acetylcholine expression, thereby delaying colon cancer progression.

Conclusions • This study suggests that mustard oil's modulation of acetylcholine expression within the internal environment holds the potential for impeding colon cancer growth. (*Altern Ther Health Med.* 2023;29(8):246-251).

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INTRODUCTION

Acetylcholine (ACh) is a vital neurotransmitter in the human body to enhance memory, promote gastrointestinal movement, and regulate glandular secretion. Numerous studies have highlighted the intricate connection between ACh and the emergence and progression of malignant tumors,[1-2] an area of research that continues to evolve. A widely accepted notion is that ACh can act as a molecular signal, binding to its corresponding receptors to stimulate tumor growth.[3] This phenomenon has been confirmed across various cancer types, including gastric cancer,[4] colorectal cancer,[5] lung cancer,[6] and breast cancer.[7] As our understanding of the ACh-tumor relationship deepens, it becomes evident that ACh's impact on tumor growth extends beyond this receptor-mediated mechanism. Recent research underscores the

expanding role of the human nervous system as a regulator of tumor development within the internal environment,[8-9] wherein ACh assumes a pivotal role as a neurotransmitter.

ACh exerts a crucial influence on tumor growth and metastasis by altering the internal tumor environment. Recent studies have unveiled ACh's ability to impact the human immune system, fostering tumor progression. ACh operates by modulating immune cell functions, consequently influencing tumor immune evasion and surveillance processes.[10-11] These discoveries underscore the significant role of ACh as a neurotransmitter within the tumor milieu, capable of orchestrating diverse mechanisms to influence tumor growth. However, depending only on blocking cholinergic receptors might be insufficient to stop tumors from growing. Furthermore, the multifarious sources of ACh secretion pose a formidable challenge in attempting to intercept its release.

Considering the above information, our study aimed to explore the impact of reducing ACh levels in the body to slow down tumor growth. We performed experiments using colon cancer cells closely affected by ACh and its effects on their growth.

MATERIALS AND METHODS

Cell Culture and Experimental Setup

HCT116 and LoVo cells, procured from the Cell Resource Center at the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, were cultured in RPMI-1640 media (ThermoFisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (FBS). The cell lines were cultivated in an incubator maintained at 37°C with a 5% CO_2 atmosphere.

Cell Seeding and ACh Concentration Gradients

A specific quantity of HCT116 and LoVo cells, cultivated in their logarithmic growth phase, were evenly distributed in 96-well plates. Six concentration gradients (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} mol/L) of ACh-containing medium and blank medium were introduced into separate wells.

Incubation and CCK-8 Assessment

After 72 hours, 10 μL of CCK8 reagent was added to each well. The plate was then incubated at 37°C for 2-3 hours to halt the culture process. This incubation also included the addition of 1% fetal bovine serum to the medium.

Preparation of ACh Chloride Concentrations

ACh chloride concentrations were prepared by dissolving slightly over 186mg of ACh chloride powder in 10mL of 1640 medium, yielding a 10^{-1} mol/L solution. Following filtration, subsequent dilutions generated concentration levels spanning 10^{-2} to 10^{-7} mol/L.

Measurement of Cell Proliferation

The optical density (OD value) of each well was assessed at 450 nm using a microplate reader. The corresponding OD values were then employed to quantify the extent of cell proliferation.

Tumor Implantation Protocol

Colon cancer cells have the capability to elevate the Ach concentration within mouse serum through an autocrine mechanism. For this study, we employed non-immune deficient Balb/c mice and CT26 murine colon cancer cells. The mice were randomly divided into two groups: experimental and control groups, each containing 14 mice. Subsequently, CT26 colon cancer cells in their logarithmic growth phase were subcutaneously implanted into the experimental group mice at a specific concentration. Experimental and control group mice were provided standard feed for two weeks after tumor formation. After this period, blood samples were collected from the eyeballs, and serum ACh concentrations were assessed using the Elisa method.

Mustard Oil Intervention in Murine Model

A total of 28 6-week-old Balb/c mice, matched in size and weight, were randomly allocated into two groups: an experimental group and a control group comprising 14 mice. The control group mice were given feed containing soybean oil (accounting for 15% of the feed composition), while the experimental group mice were given feed containing mustard oil (with 3% mustard oil constituting 15% of the feed).

Following two weeks, ACh expression levels were assessed using the Elisa method. Blood samples were collected through eyeball sampling from both groups of mice.

Impact of Mustard Oil on ACh Expression in Colon Cancer-Bearing Mice

Intriguingly, we investigated the influence of mustard oil on ACh expression in mice bearing colon cancer. CT26-derived colon cancer cells were implanted beneath the skin of Balb/c mice. Once successful inoculation was achieved, the colon cancer-bearing mice were arbitrarily divided into an experimental and control group comprising 14 mice. The experimental group received a diet enriched with mustard oil, while the control group was fed a soybean oil diet. Two weeks later, serum ACh expression in the two groups of colon cancer-bearing mice was evaluated using the Elisa method.

RESULTS

Proliferation Response to Varied Acetylcholine Concentrations

The results indicated a notable trend wherein cell proliferation gradually increased with rising ACh concentrations in the medium, from normal levels to 10^{-5}mol/L ACh. Notably, the highest proliferation rate was observed at 10^{-5}mol/L, with a statistically significant difference. Subsequently, as ACh concentration increased beyond this point, cell proliferation exhibited a gradual decline, even falling below the proliferation rate of cells in the normal ACh-free medium. This decline could potentially be attributed to the cytotoxic effects stemming from excessive ACh concentration, a hypothesis requiring validation through further experimentation.

Table 1. The proliferation rate of colon cancer HCT116 and LoVo cells at different ACh concentrations

Group\ACh concentration	without	10^{-7} mol/L	10^{-6} mol/L	10^{-5} mol/L	10^{-4} mol/L	10^{-3} mol/L	10^{-2} mol/L	*F*	*P* value
OD Value in HCT116 cell	1.01 ± 0.51	1.09 ± 0.28	1.24 ± 0.24	1.68 ± 0.53	1.01 ± 0.50	0.86 ± 0.32	0.62 ± 0.25	6.921	.000
OD Value in LoVo cell	0.91 ± 0.37	0.99 ± 0.33	1.02 ± 0.34	1.53 ± 0.62	1.15 ± 0.45	0.89 ± 0.33	0.68 ± 0.23	4.509	.001

Abbreviations: ACh, acetylcholine; OD, optical density. The table presents the proliferation rate of colon cancer HCT116 and LoVo cells at various acetylcholine concentrations (ACh). Optical density (OD) values were measured for HCT116 and LoVo cells in response to different ACh concentrations ranging from 10^{-7} mol/L to 10^{-2} mol/L.

Promotion of Colon Cancer Cell Proliferation by ACh

The experimental findings demonstrated that a specific concentration of ACh played a role in promoting the proliferation of colon cancer HCT116 and LoVo cells, refer to Figure 1. Detailed proliferation rates for colon cancer HCT116 and LoVo cells across different ACh concentrations are presented in Table 1.

Autocrine Increase of Acetylcholine Concentration in Mouse Serum by Colon Cancer Cells

The results revealed significant variations in the relative expression levels of Ach, with tumor-bearing Balb/c mice serum exhibiting levels of 402.16 ± 40.51, in contrast to normal Balb/c mice serum levels of 362.91 ± 34.79. Statistical analysis indicated a significant disparity ($t = 2.750$, $P = .011$) between the two groups (see Figure 2). Given the consistent experimental conditions, we speculate that the observed elevation in serum ACh concentration in tumor-bearing Balb/c mice is a consequence of the autocrine effect of colon cancer cells.

Mustard Oil Effectively Decreases Serum ACh Expression in Normal Balb/c Mice

The experimental findings revealed a significant reduction in serum ACh expression concentration among the experimental group mice (339.56 ± 25.78) when compared to the control group mice (367.39 ± 23.17) ($P = .006$), refer to Figure 3. This finding suggests that mustard oil effectively decreased the concentration of ACh expression in normal Balb/c mice.

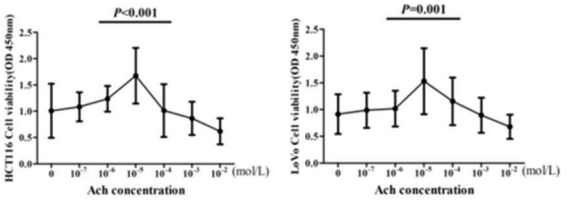
Serum ACh Expression Assessment

Notably, the findings highlighted a substantial reduction in ACh expression within the serum of the mustard oil-treated tumor-bearing mice group (373.20 ± 31.85) in comparison to the control group (415.08 ± 38.35), with the distinction being statistically significant ($t = 3.144$, $P = .004$), refer to Figure 4).

Tumor Characterization and Analysis

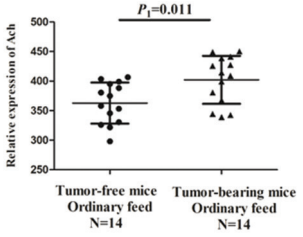
Upon completion of the experiment, all mice were humanely sacrificed, and subcutaneous tumors were excised for subsequent analysis. Tumor dimensions were meticulously measured utilizing a vernier caliper. Subsequently, tumor masses were preserved in a 10% formalin solution, followed by standard wax embedding procedures and HE staining. Notably, HE staining confirmed the identity of all tissue specimens as colon cancer tissue (refer to Figure 5).

Figure 1. Cell proliferation rate in different concentrations of ACh.



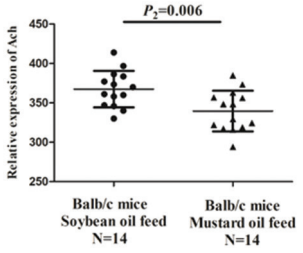
Note: The figure illustrates the impact of mustard oil on serum ACh expression concentration in tumor-bearing Balb/c mice. The experimental group received mustard oil feed, while the control group was fed soybean oil. Elisa method was used to measure ACh expression levels in serum samples from both groups.

Figure 2. Expression of ACh in colon tumor-bearing mice and tumor-free mice.



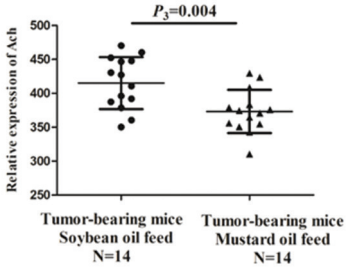
Note: The figure depicts the expression of acetylcholine (ACh) in two distinct groups: colon tumor-bearing mice and tumor-free mice. The figure visually represents the relative ACh expression levels between these groups.

Figure 3. Expression of ACh in normal Balb/c mice with different diets.



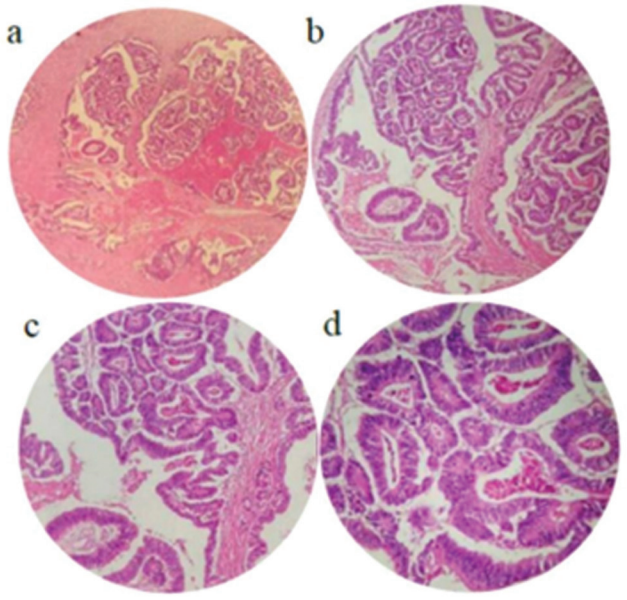
Note: Figure 3 illustrates the expression of acetylcholine (ACh) in normal Balb/c mice subjected to different diets. The figure visually represents the variation in ACh expression levels among mice fed soybean oil (control group) and those fed with mustard oil (experimental group). The comparison between these dietary conditions provides valuable insights into the influence of different diets on ACh expression.

Figure 4. Expression of ACh in colon tumor-bearing mice with different diets.



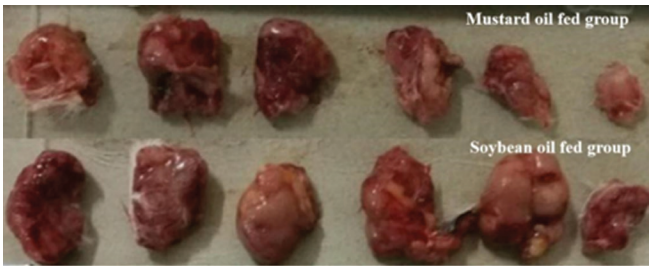
Note: Figure 4 showcases the expression of acetylcholine (ACh) in colon tumor-bearing mice subjected to distinct diets. The figure visually portrays the variations in ACh expression levels between mice fed with soybean oil (control group) and those fed with mustard oil (experimental group).

Figure 5. HE staining of colon cancer in tumor-bearing mice (a:×50, b:×100, c:×200 and d: ×400).



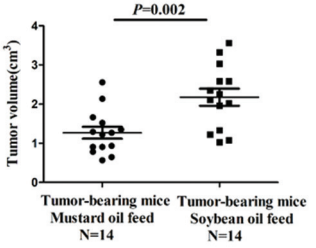
Note: the figure presents histopathological images of colon cancer in tumor-bearing mice, showcasing different magnifications. The images captured at ×50, ×100, ×200, and ×400 magnifications (a, b, c, and d, respectively) provide detailed visualizations of the tumor tissue morphology. Hematoxylin and eosin (HE) staining was employed to enhance tissue contrast, revealing cellular and structural characteristics of colon cancer.

Figure 6. Colon cancer tissue of tumor-bearing mice fed with mustard oil and soybean oil



Note: The figure compares colon cancer tissue from tumor-bearing mice subjected to different diets: the mustard oil group (a) and the soybean oil group (b). The figure visually contrasts the tissue characteristics between these two dietary conditions. The use of mustard oil and soybean oil as dietary factors offers insights into potential variations in the tumor microenvironment.

Figure 7. Colon cancer tissue of tumor-bearing mice fed with mustard oil and soybean oil



Note: a: Mustard oil group b: soybean oil group.

Effect on Tumor Morphology and Volume

Remarkably, the results underscored a significant variance in tumor tissue between the soybean oil and mustard oil groups. Tumors in the soybean oil group appeared notably enlarged and predominantly irregular in shape. In contrast, the mustard oil group exhibited predominantly oval tumor shapes, accompanied by markedly smaller tumor volumes (1.27 ± 0.56) in contrast to the soybean oil group (2.18 ± 0.81). This dissimilarity was statistically significant ($t = 3.43$, $P = .002$); refer to Figure 6 and Figure 7, indicating that mustard oil intervention may contribute to reduced tumor growth and distinct morphological features.

DISCUSSION

The influence of ACh in promoting the emergence and advancement of diverse tumors, particularly in colorectal cancer, has garnered substantial attention.[12] Studies have revealed the pronounced overexpression of M_3 muscarinic receptor (M_3R) mRNA and protein within colon cancer. ACh operates via M3R to actively facilitate the invasion and migration of tumor cells, a phenomenon underscored by existing research.[13] However, while strides have been made in this realm of investigation, the practical application of these findings has encountered limitations, impeding significant advancement.

Our study extends beyond the established pathways through which ACh potentially influences tumor development. We propose that ACh's impact might encompass uncharted pathways, contributing to its multifaceted influence on tumorigenesis. For instance, a study by Hayakawa et al.[9] illuminates an intriguing avenue: cholinergic stimulation of the gastric epithelium triggers the expression of nerve growth factor (NGF), thereby eliciting NGF overexpression within the gastric epithelium. This complex process, in turn, promotes the expansion of enteric nerves, ultimately promoting carcinogenesis.

As researchers delve into the intricacies of the tumor microenvironment, they have identified the autonomic nervous system as a crucial component with a notable influence on regulating tumor development. Monje et al.[8] have contributed insights into this realm, emphasizing the pivotal role of NGF secretion by colon cancer cells. This secretion initiates cholinergic innervation within the tumor microenvironment, setting the stage for intricate interactions. ACh, subsequently released from invading nerves, propels cancer cell proliferation by upregulating Wnt signaling pathways.

The remarkable success of immune checkpoint inhibitors in cancer treatment has heightened the focus on immune system research. ACh has emerged as a critical player in this context. Research has revealed that T cells within the spleen release ACh when stimulated by specific signals. This ACh then engages with relevant receptors on macrophages, contributing to immune activity,[14-15] though certain aspects of this immune function still require further explanation.[16]

In the colonic environment, ACh originates from a diverse array of sources, encompassing the release of vagus nerve terminals, hepatic regulation,[17] tuft cell secretion within the intestinal lining,[18] and the autocrine activity of tumor cells[19] as supported by our study results. Additionally, these secretory pathways can be influenced by emotional states, dietary factors, and more. The complex interaction of multiple pathways and factors leads to changes in *in-vivo* levels of ACh, which directly affects tumor growth rate. The findings of this study underscore the connection between varying ACh concentrations in the growth milieu and the proliferation rate of colon cancer cells. It suggests that adjusting ACh levels in the body could be a way to slow down tumor growth. The findings accentuate the potential avenue for novel therapeutic interventions in cancer management.

Drawing insights from traditional Chinese medicine, we investigated the possible connection between semen brassicae and the inhibition of ACh expression within the human body. Consequently, we selected mustard oil for our experiment. The outcomes validated our hypothesis, revealing a significant reduction in ACh expression concentration in the serum of regular mice following mustard oil administration. Moreover, our findings align with prior research that underscores mustard oil's notable inhibitory effects on a spectrum of tumors.[20-24] Specifically, this study contributes evidence showcasing mustard oil's efficacy in impeding colon cancer growth. Notably, mice administered with mustard oil exhibited lower serum ACh expression levels, reinforcing that mustard oil's potential to curtail colon cancer growth stems from its capacity to optimize the tumor's internal environment and diminish ACh expression.

Study Limitations

This study, while providing valuable insights, has certain limitations. The focus primarily on mustard oil's effect on ACh expression in serum leaves unexplored the potential influence of ACh in other bodily fluids like intestinal fluid. Additionally, the specific mechanisms through which mustard oil affects ACh expression require further investigation, including whether it directly impacts ACh or acts through specific enzymes. The small sample size of mice, chosen due to ACh's short lifespan, could limit the generalizability of findings. This preliminary study's exploratory nature suggests the need for more comprehensive investigations. Factors such as emotional states and diet influencing ACh were not accounted for, potentially affecting outcomes. While promising, the application of mustard oil for tumor growth control requires further clinical validation, considering potential long-term effects and its applicability to human responses.

CONCLUSION

In conclusion, this study sheds light on the potential of mustard oil to modulate ACh expression and its impact on tumor growth. The observed reduction in ACh concentration within serum and its association with slowed tumor growth highlights a promising avenue for future research. While the findings offer initial insights, further investigations are warranted to unravel the intricate mechanisms underlying ACh modulation and its applicability as a therapeutic strategy. This preliminary exploration serves as a stepping stone toward advancing our understanding of ACh's role in cancer progression and the potential implications of mustard oil in tumor growth control.

CONFLICT OF INTEREST

The authors declare to have no conflict of interest.

FUNDING

This study did not receive funding in any form.

DATA AVAILABILITY STATEMENT

Supporting data is available upon request from corresponding author.

AUTHOR CONTRIBUTIONS

Hu Chen and Na Wang contributed equally and are considered co-first authors.

REFERENCES

1. Grando SA. Connections of nicotine to cancer. *Nat Rev Cancer*. 2014;14(6):419-429. doi:10.1038/nrc3725
2. Aronowitz AL, Ali SR, Glaun MDE, Amit M. Acetylcholine in carcinogenesis and targeting cholinergic receptors in oncology (adv. biology 9/2022) [J/OL]. *Adv Biol*. 2022;6(9):2270091. doi:10.1002/adbi.202270091
3. Chen, J; Cheuk, IWY; Shin, VY; et al. Acetylcholine receptors: Key players in cancer development. Surg Oncol.2019,31():46-53
4. Wang L, Xu J, Xia Y, et al. Muscarinic acetylcholine receptor 3 mediates vagus nerve-induced gastric cancer. *Oncogenesis*. 2018;7(11):88. doi:10.1038/s41389-018-0099-6
5. Hering NA, Liu V, Kim R, et al. Blockage of Cholinergic Signaling via Muscarinic Acetylcholine Receptor 3 Inhibits Tumor Growth in Human Colorectal Adenocarcinoma. *Cancers (Basel)*. 2021;13(13):3220. doi:10.3390/cancers13133220
6. Young RP, Scott RJ. Inhaled nicotine and lung cancer: potential role of the nicotinic acetylcholine receptor. *Proc Natl Acad Sci USA*. 2020;117(9):4460-4461. doi:10.1073/pnas.1921567117

7. Sun Z, Bao J, Zhangsun M, Dong S, Zhangsun D, Luo S. αO-Conotoxin GeXIVA Inhibits the Growth of Breast Cancer Cells via Interaction with α9 Nicotine Acetylcholine Receptors. *Mar Drugs*. 2020;18(4):195. doi:10.3390/md18040195
8. Monje M. Settling a Nervous Stomach: The Neural Regulation of Enteric Cancer. *Cancer Cell*. 2017;31(1):1-2. doi:10.1016/j.ccell.2016.12.008
9. Slominski RM, Raman C, Chen JY, Slominski AT. How cancer hijacks the body's homeostasis through the neuroendocrine system. [J/OL]. *Trends Neurosci*. 2023;46(4):263-275. doi:10.1016/j.tins.2023.01.003
10. Cox MA, Bassi C, Saunders ME, et al. Beyond neurotransmission: acetylcholine in immunity and inflammation. *J Intern Med*. 2020;287(2):120-133. doi:10.1111/joim.13006
11. Wang, Z; Liu, W; Wang, C; et al. Acetylcholine promotes the self-renewal and immune escape of CD133+ thyroid cancer cells through activation of CD133-Akt pathway. Cancer Lett.2020,471():116-124
12. Shi Y, Luo J, Wang X, et al. Emerging Trends on the Correlation Between Neurotransmitters and Tumor Progression in the Last 20 Years: A Bibliometric Analysis *via* CiteSpace. *Front Oncol*. 2022;12:800499. doi:10.3389/fonc.2022.800499
13. Kuol N, Davidson M, Karakkat J, et al. Blocking Muscarinic Receptor 3 Attenuates Tumor Growth and Decreases Immunosuppressive and Cholinergic Markers in an Orthotopic Mouse Model of Colorectal Cancer. *Int J Mol Sci*. 2022;24(1):596. doi:10.3390/ijms24010596
14. Han B, Li X, Hao J. The cholinergic anti-inflammatory pathway: an innovative treatment strategy for neurological diseases. *Neurosci Biobehav Rev*. 2017;77(7):358-368. doi:10.1016/j.neubiorev.2017.04.002
15. Cox MA, Bassi C, Saunders ME, et al. Beyond neurotransmission: acetylcholine in immunity and inflammation. *J Intern Med*. 2020;287(2):120-133. doi:10.1111/joim.13006
16. Malin SG, Shavva VS, Tarnawski L, Olofsson PS. Functions of acetylcholine-producing lymphocytes in immunobiology. *Curr Opin Neurobiol*. 2020;62(8):115-121. doi:10.1016/j.conb.2020.01.017
17. Mikami Y, Tsunoda J, Kiyohara H, Taniki N, Teratani T, Kanai T. Vagus nerve-mediated intestinal immune regulation: therapeutic implications of inflammatory bowel diseases. *Int Immunol*. 2022;34(2):97-106. doi:10.1093/intimm/dxab039
18. Hendel SK, Kellermann L, Hausmann A, Bindslev N, Jensen KB, Nielsen OH. Tuft Cells and Their Role in Intestinal Diseases. *Front Immunol*. 2022;13:822867. doi:10.3389/fimmu.2022.822867
19. Cheng K, Samimi R, Xie G, et al. Acetylcholine release by human colon cancer cells mediates autocrine stimulation of cell proliferation. *Am J Physiol Gastrointest Liver Physiol*. 2008;295(3):G591-G597. doi:10.1152/ajpgi.00055.2008
20. Bhattacharya A, Li Y, Wade KL, Paonessa JD, Fahey JW, Zhang Y. Allyl isothiocyanate-rich mustard seed powder inhibits bladder cancer growth and muscle invasion. *Carcinogenesis*. 2010;31(12):2105-2110. doi:10.1093/carcin/bgq202
21. Núñez-Iglesias MJ, Novío S. Glucosinolate-Degradation Products as Co-Adjuvant Therapy on Prostate Cancer in Vitro. 2019;20(20). doi:10.3390/ijms20204977
22. Rakariyatham K, Yang X, Gao Z, et al. Synergistic chemopreventive effect of allyl isothiocyanate and sulforaphane on non-small cell lung carcinoma cells. *Food Funct*. 2019;10(2):893-902. doi:10.1039/C8FO01914B
23. Qin G, Li P, Xue Z. Effect of allyl isothiocyanate on the viability and apoptosis of the human cervical cancer HeLa cell line *in vitro*. *Oncol Lett*. 2018;15(6):8756-8760. doi:10.3892/ol.2018.8428
24. Jiang, Z; Liu, X; Chang, K; et al. Allyl Isothiocyanate Inhibits the Proliferation of Renal Carcinoma Cell Line GRC-1 by Inducing an Imbalance Between Bcl2 and Bax. Med Sci Monit.2016,22():4283-4288.