Journal of Molecular Histology (2025) 56:136
https://doi.org/10.1007/s10735-025-10409-3

ORIGINAL PAPER

The effects of ozone exposure on apoptotic mechanisms in Colo-320 and Colo-741 cell lines: implications for cancer therapy

Remziye Kendirci-Katirci[1] · Ertan Katirci[2] · Tuna Onal[3] · H. Seda Vatansever[4,5]

Received: 8 December 2024 / Accepted: 21 March 2025 / Published online: 18 April 2025

Abstract

This study aimed to investigate the effects of ozone exposure on apoptotic mechanisms in primary (Colo-320) and metastatic (Colo-741) cell lines. Colo-320 and Colo-741 cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and treated with 20 µg/mL ozone for 72 h after MTT assay. Inverted microscopy was used for morphological assessment, and immunocytochemistry followed by H-SCORE analysis was performed to measure the expression of apoptotic markers. The statistical analysis was conducted using unpaired T-tests or Mann–Whitney U tests, with significance set at $p<0.05$. Morphological analysis showed no significant changes in cell shape in Colo-320 or Colo-741 cells after ozone exposure. However, immunocytochemical analysis revealed significant increases in semi-quantitative histological scores (H-SCORES) for cleaved caspase-3, Bax, and cytochrome c in both cell lines, indicating enhanced apoptotic activity ($p<0.05$). Conversely, Bcl-2 expression was significantly decreased in both Colo-320 and Colo-741 cell lines after ozone exposure ($p<0.05$). Ozone exposure promoted apoptosis in both Colo-320 and Colo-741 cell lines, as evidenced by increased pro-apoptotic markers and decreased anti-apoptotic markers. These molecular changes were notable, yet they did not visibly alter cell morphology. The observed similarities between Colo-320 and Colo-741 cell responses suggest the need for further investigation. These findings indicate that ozone exposure may influence tumor cell apoptosis while preserving cell structure, highlighting the necessity of further research into its potential therapeutic implications.

Keywords Colo-320 · Colo-741 · Ozone exposure · Apoptosis · Cell morphology

Introduction

Colorectal adenocarcinoma is characterized by uncontrolled growth of glandular epithelial cells in the colon or rectum. In the treatment of this malignancy, the main treatment options, i.e., surgery, radiation, and chemotherapy are routinely ineffective for patients with metastatic disease, because the side-effects are high, as the treatment strategies are not tumour-specific and difficult to discriminate normal and cancer cells (Noh et al. 2020). Recent advancements in immunotherapy for treating some solid tumors, including colorectal cancer have shown promise, but, considering the terminal prognosis for patients with metastatic disease, effective new therapies with admirable tumour-targeting selectivity and effectiveness are still desperately needed (Angerilli et al. 2021; Liu et al. 2022).

The application of ozone therapy within the realm of oncology exists as an element of a more expansive array of medical utilizations. This therapeutic modality has been utilized in addressing dermatological pathologies, intervertebral disc protrusions, complications stemming from diabetes, conditions of the oral mucosa, as well as cardiovascular and cerebrovascular ailments, thus underscoring its multifaceted nature in clinical practice (Liu et al. 2023). Empirical investigations have demonstrated that ozone

✉ H. Seda Vatansever
sedavatansever@yahoo.com

1 Department of Histology and Embryology, School of Medicine, Akdeniz University, Antalya, Turkey

2 Department of Histology and Embryology, School of Medicine, Kirsehir Ahi Evran University, Kirsehir, Turkey

3 Department of Histology and Embryology, School of Medicine, Bandirma Onyedi Eylul University, Balikesir, Turkey

4 Department of Histology and Embryology, School of Medicine, Manisa Celal Bayar University, Manisa, Turkey

5 DESAM Research Institute, Near East University, Mersin 10, Turkey

therapy may serve as a beneficial adjunctive intervention for patients afflicted with neoplasms who encounter fatigue, applicable both in the course of oncological treatment and within palliative scenarios, notably devoid of considerable adverse effects (Li and Pu 2024; Tirelli et al. 2018). Moreover, the implementation of ozone therapy has been effectively employed for the alleviation of symptoms in patients with malignancies, thereby illustrating its prospects in the management of symptoms associated with cancer (Clavo et al. 2023).

Ozone exposure has attracted great interest in various medical fields for its beneficial effects such as cancer treatment. Studies have revealed that ozone exposure induces apoptosis in various cancer cell lines through activation of both intrinsic and extrinsic apoptotic pathways. For example, studies have shown that ozone exposure can lead to an increase in the Bax/Bcl-2 ratio and promote apoptosis by

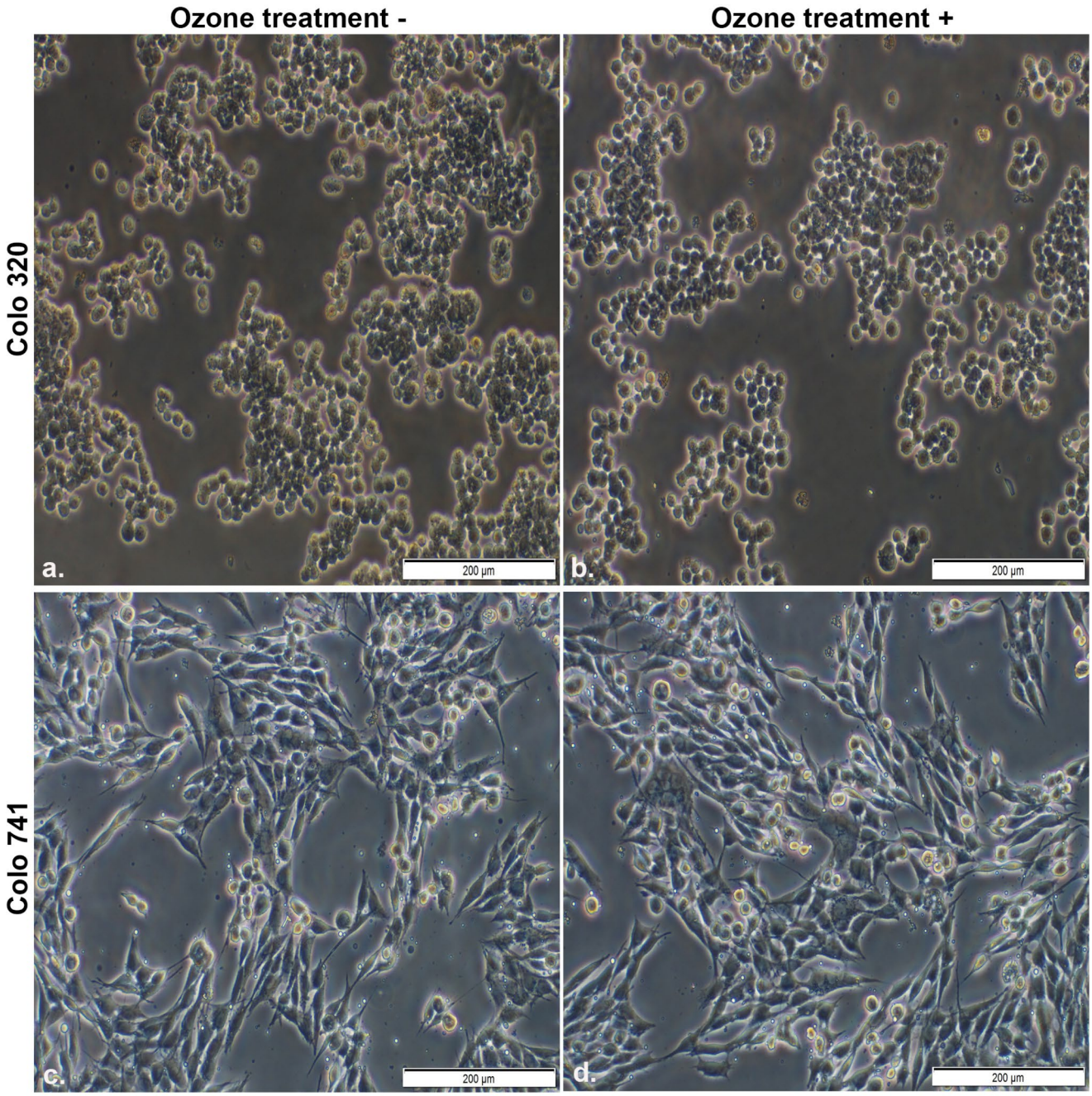

Fig. 1 Colo-320 and Colo-741 cells imaged under the inverted microscope: **a** Colo-320 cells, **b** ozone-exposured Colo-320 cells **c** Colo-741 cells, **d** ozone-exposured Colo-741 cells. Scale bars = 200 µm

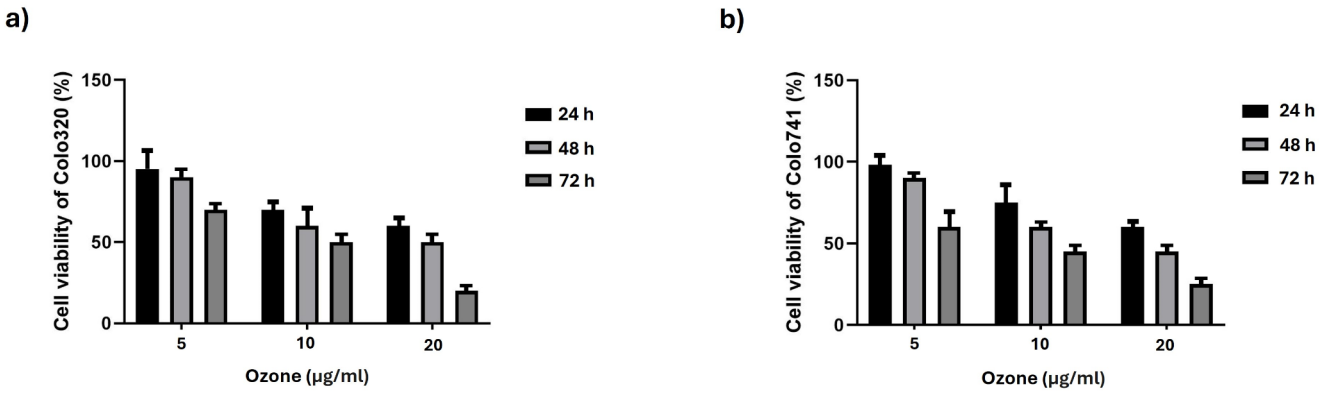

Fig. 2 Cell viability in **a** Colo-320 and **b** Colo-741 cells at different concentrations and times. The data are presented as the mean ± SD

facilitating the release of cytochrome c from mitochondria into the cytosol (Cai et al. 2020; Xu et al. 2021). Cytochrome-c release leads to the activation of caspase-9 and caspase-3, respectively, leading to the initiation of apoptosis (Rivas-Arancibia et al. 2015; Tang et al. 2021). In colorectal adenocarcinoma, apoptotic markers are associated with tumour progression and disease prognosis. Previous studies have reported that decreases in the level of anti-apoptotic protein (Bcl-2) and increases in the level of pro-apoptotic protein (Bax) are associated with increased apoptosis in colorectal cancer cells (Aydos et al. 2014). The effects of ozone exposure on colorectal adenocarcinoma have not yet been fully addressed, most importantly in terms of modulation of apoptotic pathways. This research aims to address the gap in this field by clarifying the effect of ozone exposure on colorectal adenocarcinoma cell lines primary (Colo-320) and metastatic (Colo-741), focusing on major apoptotic markers such as cleaved caspase-3, Bax, Bcl-2 and cytochrome c. Understanding how ozone affects these markers may provide valuable insights into its potential as a complementary therapy in oncology.

Methods

Cell culture

Colo-320 (primary human colon adenocarcinoma, ATCC catalog: CCL 220) and Colo-741 cell lines (metastatic human colon adenocarcinoma, ECACC 93052621) were cultured in RPMI-1640 media (Biochrom, FG1215) containing 10% FBS (Capricorn Scientific, FBS-11B), 1% penicillin-streptomycin (Biochrom, A2213), and 1% L-glutamine (Özsoy et al. 2020). Cells were grown in a humid environment with 5% CO^2 at 37 °C. When the cells reached 80% confluence, they were routinely subcultured using 0.25% trypsin-EDTA solution (Biochrom L2143).

Cell viability—MTT assay

Colo320 and Colo741 cell lines were seeded in 96-well plates at a concentration of 5×10^4 cells/well (Özsoy et al. 2020). Cells were treated with ozone at concentrations of 5-10-20 µg/ml for 24, 48, 72 h at 37 °C in a high humidity incubator (Yıldırım et al. 2022). MTT solution (Biotium, #30006) was heated to 37 °C, then 10 µl of solution was added to each well. Cells were incubated at 37 °C and 5% CO^2 for 4 hours. Then 200 µl DMSO was added in each well to block crystallising formazan salts. Absorbance was measured at 570 nm with a spectrophotometer (Versa Max, Molecular Device, Sunnyvale, USA).

Ozone exposure

In the experiments applied to Colo-320 and Colo-741 cell lines, ozone exposure was performed at concentrations of 20 µg/mL in isotonic sodium chloride solution. This treatment was applied for 72 h to evaluate the exposure of cells to ozone and the associated cellular responses (Yıldırım et al. 2022). For comparison, cells that were not exposed to ozone were established as the control group and maintained under the same culture conditions.

Immunocytochemistry

Cultures were also evaluated for the binding of antibodies against Distribution of cleaved-caspase-3, Bax, Bcl-2 and cytochrome-c were evaluated immunocytochemically in

both Colo-320 and Colo-741 cells. Cells from all groups were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 30 min at 4 °C. To facilitate permeabilization of the cells, 0.01 Tween 20 (P1379, Sigma Aldrich) was added on ice and kept for 15 min. After washing with PBS, endogenous peroxidase activity was inhibited by incubating with 3% H_2O_2 (107209, Merck) for 5 min at room temperature. Cells were then washed with PBS and treated with primary antibodies against anti-cleaved-caspase-3 (#9661, Cell Signalling), anti-Bax (A12539, Abcam), anti-Bcl-2 (A86278, Abcam), and anti-cytochrome-c (A88286, Abcam) overnight at 4 °C. Secondary antibodies were applied using the Histostain-Plus IHC Kit (HRP, #859043, ThermoFisher Scientific), following the manufacturer's instructions. Briefly, after primary antibody incubation, cells were incubated with a universal secondary antibody (anti-rabbit/mouse IgG) conjugated to horseradish peroxidase (HRP) for 1 h at room temperature. This step included endogenous protein blocking with the kit's proprietary blocking solution to minimize nonspecific binding. After washing, immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB, 8059, Cell Signaling) substrate, which produces a brown precipitate upon HRP-catalyzed oxidation. Cells were counterstained with Mayer's hematoxylin to highlight nuclei. After, they were mounted using mounting media (Merck Millipore 107961, Germany). All samples were then examined using a light microscope (Olympus BX40, Tokyo, Japan).

Cleaved-caspase-3, Bax, Bcl-2 and cytochrome-c staining were also semi-quantitatively graded using the H-SCORE calculated by the following equation: HSCORE = Σл (i + 1), where i is the staining intensity with a value of 1, 2 or 3 (mild, moderate or strong, respectively) and л is the percentage of cells stained at each intensity ranging from 0 to 100% (Becer et al. 2019).

Statistical analysis

Means, standard deviations and medians of the experimental data were calculated using descriptive statistics and the data were presented as mean ± SD. Quantitative data were subjected to normality tests with Shapiro-Wilk normality test. Data were compared using the unpaired T test followed by the Holm-Sidak post hoc test for data passing the normality test, otherwise, the Mann–Whitney U test followed by the Dunn test was used (GraphPad Prism 9). $p < 0.05$ between different groups was considered statistically significant.

Fig. 3 Immunoreactivity of Cleaved Caspase-3, Bax, Bcl-2 and Cytochrome c in Colo-320 for 72 h culture with standard culture conditions or 20 µg/mL ozone exposure. Scale bars = 20 µm

Results

Cell morphology

Colo-320 cells showed typical epithelioid colony morphology (Fig. 1a). After 72 h of ozone exposure, the cell morhology of Colo-320 cells were not affected (Fig. 1a, b). Colo-741 cells were fibroblast-like cells with typical fibroblast colony morphology (Fig. 1c). It was noted that the cell morhology of Colo-741 cells were not affected after 72 h of ozone exposure (Fig. 1c, d).

Cell viability and toxicity

The effect of ozone treatment at different concentrations (5–20 µg/ml) on the viability of Colo320 and Colo741 cells was determined for 24, 48 and 72 h (Fig. 2). The greatest decrease in cell viability was observed at the dose of 20 µg/mL at 72 h incubation time (more than 50% decrease in cell viability). At lower concentrations and incubation times, the decrease in cell viability did not even reach 50%.

Immunocytochemical analysis

To analyse the effect of ozone exposure on the Colo-320 cell line, we performed immunocytochemical and semi-quantitative analysis evaluation for cleaved-caspase-3, Bax, Bcl-2 and cytochrome-c proteins (Fig. 3). There were a significant increase intensities for cleaved-caspase-3, Bax and cytochrome-c after ozone exposure ($p < 0,05$) (Table 1). Specifically, cleaved-caspase-3 and Bax levels were significantly higher in the ozone-treated group compared to the control group, reflecting increased apoptotic activity ($p < 0,05$). Similarly, cytochrome-c immunostaining was significantly increased in ozone-treated cells as a reflection of the apoptotic mechanism ($p < 0,05$). Conversely, Bcl-2 immunostaining was decreased in ozone-treated cells compared to untreated cells ($p < 0,05$) (Table 1). This decrease suggests that ozone exposure may prevent the anti-apoptotic function of Bcl-2 and thus promote apoptosis. Thus, these findings suggest that ozone exposure may have significant effects on apoptotic signalling in the Colo-320 cell line, decrease anti-apoptotic signals, and increase pro-apoptotic signals.

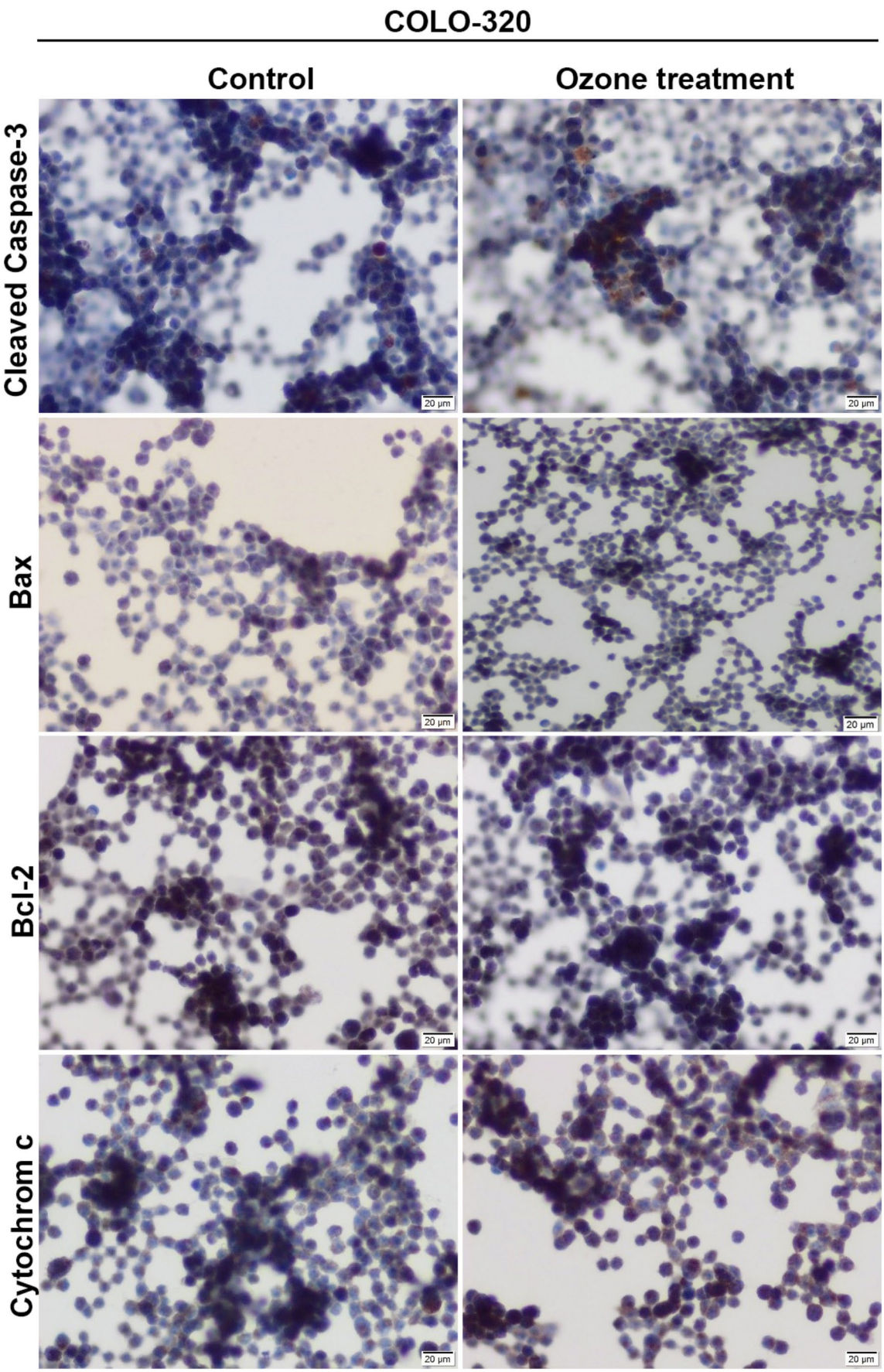
COLO-320
Control
Ozone treatment
Cleaved Caspase-3
Bax
Bcl-2
Cytochrom c
20 µm

Table 1 H-SCORE results of cleaved Caspase-3, Bax, Bcl-2 and cytochrome C proteins in Colo-320 cells treated with Ozone at a concentration of 20 µg/ml for 72 h

	Cleaved Caspase-3	Bax	Bcl-2	Cytochrome c
Colo-320	245 ± 13.23	225 ± 10	306.7 ± 5.774	281.7 ± 16.07
Colo-320 + O	290 ± 8,66[a]	358.3 ± 10.41[b]	238.3 ± 7.638[c]	370 ± 5[d]

[a,b,c,d]The data was significant when compared with Colo-320 ($p < 0.05$)

To evaluate the effect of ozone exposure on the Colo-741 cell line, we performed immunocytochemical staining and semi-quantitative analysis for cleaved caspase-3-, Bax, Bcl-2 and Cytochrome c proteins (Fig. 4). After ozone exposure, there was a significant increase in H-SCORES for cleaved caspase-3, Bax and cytochrome c, indicating strong expression of these apoptotic markers ($p < 0,05$) (Table 2). The results revealed that the ozone group exhibited significantly higher H-SCORES for cleaved caspase-3 and Bax, indicating increased apoptosis activity compared to the control group ($p < 0,05$). Furthermore, cytochrome c levels were significantly increased in ozone-treated cells compared to the control group, suggesting an important role in apoptosis ($p < 0,05$). On the other hand, Bcl-2 expression was significantly decreased in ozone-treated group compared to the untreated group ($p < 0,05$) (Table 2). This decrease suggests that ozone may lead to anti-apoptotic function of Bcl-2 and thus cell death. This suggests that ozone exposure significantly altered apoptotic responses, increasing pro-apoptotic signals and decreasing anti-apoptotic signals in the Colo-741 cell line.

Discussion

In this study, the effects of ozone exposure on cell morphology and apoptotic processes in two different primary (Colo-320) and metastatic (Colo-741) human colon adenocarcinoma cell lines were evaluated. The use of both primary and metastatic cell lines highlights whether the apoptotic responses of ozone treatment differ between primary and metastatic cancer cells.Morphological analysis of Colo-320 and Colo-741 cell lines following 72 h ozone exposure did not show significant changes in cell shape, indicating stability in cell phenotype. This is in agreement with the variability of ozone effects on cell morphology and proliferation observed in different studies (Khan 2015; Kim et al. 2003).

Fig. 4 Immunoreactivity of Cleaved Caspase-3, Bax, Bcl-2 and Cytochrome c in Colo-741 for 72 h culture with standard culture conditions or 20 µg/mL ozone exposure. Scale bars = 20 µm

The research revealed notable increases in cleaved caspase-3, Bax, and cytochrome c levels, alongside a decrease in Bcl-2 expression in both cell lines, indicating enhanced apoptotic activity (Matłok et al. 2022). These alterations suggest a shift towards apoptosis by boosting pro-apoptotic signals and suppressing anti-apoptotic signals, in line with existing literature on apoptosis regulation and ozone therapy (Xu et al. 2021). The consistent outcomes across different cell lines and previous studies underscore ozone's potential as an apoptosis-inducing agent in cancer therapy, warranting further investigation into its mechanisms and effectiveness in clinical settings (Pelinsari et al. 2024). Ozone has shown promise as a potential cancer therapy agent in the context of apoptosis regulation ability by controlling key apoptotic markers and triggering various apoptotic signal pathways (Mokoena et al. 2010). By enhancing pro-apoptotic signals like cleaved caspase-3 and Bax, and decreasing anti-apoptotic signals such as Bcl-2, ozone exposure demonstrates potential as a valuable therapeutic approach in cancer therapy (Dong et al. 2022). Further exploration is advised to delve into the detailed mechanisms of ozone-induced apoptosis to fully exploit its therapeutic potential (León Fernández et al. 2024). Given results in the literature, ozone promotes apoptosis by affecting apoptotic signals, specifically by inducing pro-apoptotic signals and causing a loss of anti-apoptotic signals, which are consistent with changes in apoptotic markers from related studies of Colo-320 and Colo-741 introduced by ozone exposure (Zapałowska et al. 2021). Ozone's potential as an apoptosis-inducing agent in cancer therapy is further reinforced by its ability to modulate apoptotic pathways, highlighting its promising role in cancer treatment (Brink et al. 2008). Ozone therapy has been demonstrated to have a wide range of actions, including immunoregulatory and anti-inflammatory properties, antioxidant activity, and the modulation of regenerative processes and epigenetic modifications (Karagülle and Yurttaş 2022). The range of these actions supports multiple functions for ozone as a primary, chemo-therapeutic agent in various medical disorders, such as cancer therapy (El-Sawalhi et al. 2012).

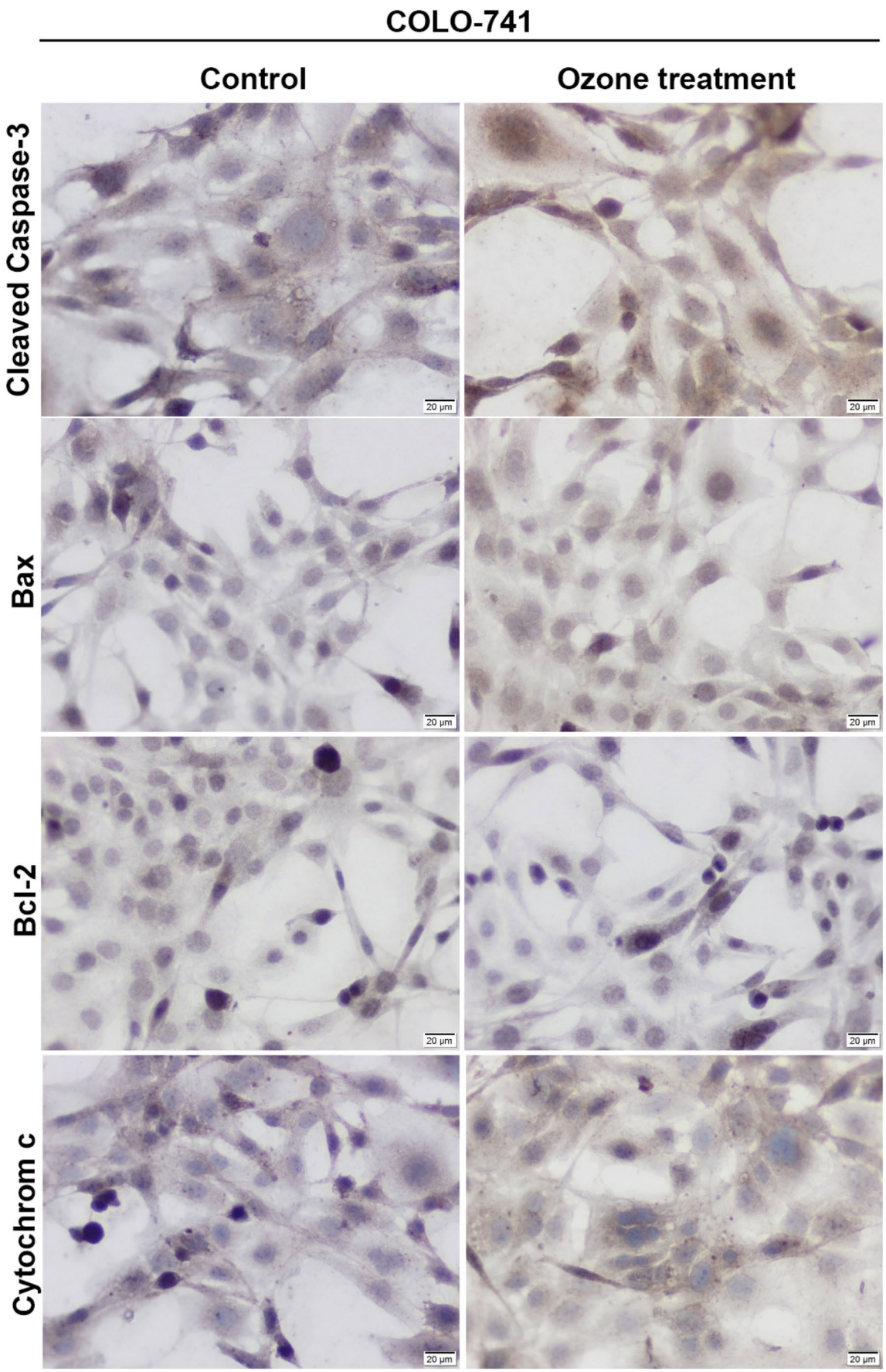
COLO-741
Control
Ozone treatment
Cleaved Caspase-3
Bax
Bcl-2
Cytochrom c
20 µm

Table 2 H-SCORE results of cleaved Caspase-3, Bax, Bcl-2 and cytochrome C proteins in Colo-741 cells treated with Ozone at a concentration of 20 µg/ml for 72 h

	Cleaved Caspase-3	Bax	Bcl-2	Cytochrome c
Colo-741	350 ± 18.03	254.5 ± 13.87	288.3 ± 17.56	301.7 ± 7.638
Colo-741 + O	390 ± 5[a]	311.7 ± 18.89[b]	251.7 ± 10.41[c]	366.7 ± 5.774[d]

[a,b,c,d]The data was significant when compared with Colo-741 ($p < 0.05$)

Conclusion

Our findings indicate that ozone exposure can affect apoptotic pathways in Colo-320 and Colo-741 cell lines. The results indicate a potential activation of pro-apoptotic signalling and inhibition of anti-apoptotic signalling, which may contribute to apoptosis induction. These findings support the need for further research into the potential role of ozone therapy in cancer treatment and its possible use in enhancing existing therapies by targeting apoptotic pathways.While this study examined the apoptotic effects of ozone on primary and metastatic colorectal adenocarcinoma cell lines, some limitations should also be considered. The isolated cell lines used do not adequately reflect the complexity of the tumour microenvironment *in vivo. In vivo*, tumours engage in dynamic interactions with surrounding cells, which may influence response to therapy and resistance mechanisms. Future research could more comprehensively evaluate the role of ozone in cancer therapy using more complex in vitro models or animal studies.

Acknowledgements We would like to thank the individuals who took part in this study.

Author contributions R.K.K.: Formal analysis, investigation, validation, visualization. E.K.: Writing-original draft. T.O.: Methodology. H.S.V.: Conceptualization, methodology, project administration, supervision, writing- review, and editing.

Funding No external funding was received for this study.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval and consent to participate This study did not require ethics approval as it involved the use of established cell lines (Colo-320 and Colo-741), which were obtained from a commercial source.

References

Angerilli V, Fontana E, Lonardi S, Sbaraglia M, Borelli B, Munari G, Salmaso R, Guzzardo V, Spolverato G, Pucciarelli S, Pilati P, Hahne JC, Bergamo F, Zagonel V, Tos D, Sadanandam AP, Loupakis A, Valeri F, N., Fassan M (2021) Intratumor morphologic and transcriptomic heterogeneity in (V600E)BRAF-mutated metastatic colorectal adenocarcinomas. ESMO Open 6(4):100211. https://doi.org/10.1016/j.esmoop.2021.100211

Aydos TR, Başar MM, Kul O, Atmaca H, Uzunalioglu T, Kısa Ü, Efe OE (2014) Effects of ozone therapy and taurine on ischemia/reperfusion-induced testicular injury in a rat testicular torsion model. Turk J Med Sci 44:749–755. https://doi.org/10.3906/sag-1308-20

Becer E, Hanoğlu DY, Kabadayı H, Hanoğlu A, Vatansever S, Yavuz DÖ, Meriçli F, Meriçli AH (2019) The effect of *Colchicum pusillum* in human colon cancer cells via Wnt/β-catenin pathway. Gene 686:213–219. https://doi.org/10.1016/j.gene.2018.11.047

Brink C, Pretorius A, Niekerk BPJ, v., Oliver DW, Venter DP (2008) Studies on cellular resilience and adaptation following acute and repetitive exposure to ozone in cultured human epithelial (HeLa) cells. Redox Rep 13(2):87–100. https://doi.org/10.1179/135100008x259187

Cai H, Xi T, Zheng L, Huang L, Peng Y, Liao R-Y, Zhu Y (2020) Ozone alleviates ischemia/reperfusion injury by inhibiting Mitochondrion-mediated apoptosis pathway in SH-SY5Y cells. Cell Biol Int 44(4):975–984. https://doi.org/10.1002/cbin.11294

Clavo B, Cánovas-Molina A, Ramallo-Fariña Y, Federico M, Rodríguez-Abreu D, Galván S, Ribeiro I, Marques da Silva SC, Navarro M, González-Beltrán D, Díaz-Garrido JA, Cazorla-Rivero S, Rodríguez-Esparragón F, Serrano-Aguilar P (2023) Effects of ozone treatment on health-Related quality of life and toxicity induced by radiotherapy and chemotherapy in symptomatic cancer survivors. Int J Environ Res Public Health. https://doi.org/10.3390/ijerph20021479

Dong X, Sun L, Ren Y, Yu X (2022) Ozone treatment increases the release of VOC from barley, which modifies seed germination. J Agric Food Chem 70(10):3127–3135. https://doi.org/10.1021/acs.jafc.1c06812

El-Sawalhi MM, Darwish HA, Mausouf MN, Shaheen A (2012) Modulation of age-related changes in oxidative stress markers and energy status in the rat heart and hippocampus: a significant role for ozone therapy. Cell Biochem Funct 31(6):518–525. https://doi.org/10.1002/cbf.2930

Karagülle OO, Yurttaş AG (2022) Ozone combined with doxorubicin exerts cytotoxic and anticancer effects on Luminal—a subtype human breast cancer cell line. Revista Da Associação Médica Brasileira 68(4):507–513. https://doi.org/10.1590/1806-9282.20211193

Khan FA (2015) Ozone induced cell death in HeLa cell culture mediated through stimulation of TNF- alpha. Moj Immunol. https://doi.org/10.15406/moji.2015.02.00051

Kim Y-H, Park H, Lim Y, Han I-O, Kwon HJ, Woods A, Oh E-S (2003) Decreased Syndecan-2 expression correlates with trichostatin-a induced-morphological changes and reduced tumorigenic activity in colon carcinoma cells. Oncogene 22(6):826–830. https://doi.org/10.1038/sj.onc.1206068

León Fernández OS, Oru GT, Viebahn-Haensler R, López Cabreja G, Espinosa S, I., Corrales Vázquez ME (2024) Medical ozone: a redox regulator with selectivity for rheumatoid arthritis patients. Pharmaceuticals 17(3):391. https://doi.org/10.3390/ph17030391

Li Y, Pu R (2024) Ozone therapy for breast cancer: an integrative literature review. Integr Cancer Ther 23:15347354241226667. https://doi.org/10.1177/15347354241226667

Liu Y, Chen L, Meng X, Ye S, Ma L (2022) Identification of hub genes in colorectal adenocarcinoma by integrated bioinformatics. Front Cell Dev Biol 10:897568. https://doi.org/10.3389/fcell.2022.897568

Liu L, Zeng L, Gao L, Zeng J, Lu J (2023) Ozone therapy for skin diseases: cellular and molecular mechanisms. Int Wound J 20(6):2376–2385. https://doi.org/10.1111/iwj.14060

Matłok N, Piechowiak T, Zardzewiały M, Balawejder M (2022) Effect of ozone treatment on the contents of selected bioactive phytochemicals in leaves of alligator plant *Kalanchoe daigremontiana*. Appl Sci 12(18):8934. https://doi.org/10.3390/app12188934

Mokoena ML, Brink C, Harvey BH, Oliver DW (2010) Appraisal of ozone as biologically active molecule and experimental tool in biomedical sciences. Med Chem Res 20(9):1687–1695. https://doi.org/10.1007/s00044-010-9493-0

Noh BJ, Kwak JY, Eom DW (2020) Immune classification for the PD-L1 expression and tumour-infiltrating lymphocytes in colorectal adenocarcinoma. BMC Cancer 20(1):58. https://doi.org/10.1186/s12885-020-6553-9

Özsoy S, Becer E, Kabadayı H, Vatansever HS, Yücecan S (2020) Quercetin-mediated apoptosis and cellular senescence in human colon cancer. Anticancer Agents Med Chem 20(11):1387–1396. https://doi.org/10.2174/1871520620666200408082026

Pelinsari SM, Sarandy MM, Vilela EF, Novaes RD, Schlamb J, Gonçalves RV (2024) Ozone exposure controls oxidative stress and the inflammatory process of hepatocytes in murine models. Antioxidants 13(2):212. https://doi.org/10.3390/antiox13020212

Rivas-Arancibia S, Zimbrón LFH, Rodríguez-Martínez E, Maldonado PD, Pérez GB, Sepúlveda-Parada M (2015) Oxidative stress-dependent changes in immune responses and cell death in the substantia Nigra after ozone exposure in rat. Front Aging Neurosci. https://doi.org/10.3389/fnagi.2015.00065

Tang S, Xu B, Li J, Zhong M, Hong Z, Zhao W, Zeng T, He X (2021) Ozone induces BEL7402 cell apoptosis by increasing reactive oxygen species production and activating JNK. Ann Transl Med 9(15):1257–1257. https://doi.org/10.21037/atm-21-3233

Tirelli U, Cirrito C, Pavanello M, Del Pup L, Lleshi A, Berretta M (2018) Oxygen-ozone therapy as support and palliative therapy in 50 cancer patients with fatigue—a short report. Eur Rev Med Pharmacol Sci 22(22):8030–8033. https://doi.org/10.26355/eurrev_201811_16432

Xu L, Zhu L, Liu P, Wu Z, Zou Z (2021) Ozone induces tolerance against cardiomyocytes oxygen–glucose deprivation/reperfusion through Inhibition of autophagy pathway. Exp Therap Med. https://doi.org/10.3892/etm.2021.10301

Yıldırım M, Erkişi S, Yılmaz H, Ünsal N, İnaç E, Tanrıver Y, Koçak P (2022) The apoptotic effect of ozone therapy on mitochondrial activity of highly metastatic breast cancer cell line MDA-MB-231 using in vitro approaches. J Interv Med 5(2):64–71. https://doi.org/10.1016/j.jimed.2022.03.004

Zapałowska A, Matłok N, Zardzewiały M, Piechowiak T, Balawejder M (2021) Effect of Ozone treatment on the quality of sea Buckthorn (*Hippophae rhamnoides* L). Plants 10(5):847. https://doi.org/10.3390/plants10050847