International Journal of Peptide Research and Therapeutics (2023) 29:100
https://doi.org/10.1007/s10989-023-10572-9

BMAP-27 Peptide Reduces Proliferation and Increases Apoptosis in Primary and Metastatic Colon Cancer Cell Lines

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Accepted: 29 September 2023 / Published online: 19 October 2023

Abstract

BMAP-27 peptide is reported to possess apoptotic and anti-proliferative effects against cancer cells but the actual mechanism of action is yet to be investigated. In the current investigation, we aimed to study the role of the BMAP-27 peptide in reducing proliferation and increasing apoptosis in colon cancer cell lines. In this study, we used primary and metastatic colon cancer cell lines SW480 and SW620. Cell proliferation was measured using MTT and CCK-8 assays, and cellular damage was analyzed by lactate dehydrogenase assay. Apoptosis, cell cycle, and proliferation potentials were measured by the expression of *CASPASE3, BAX, BCL-2, TP53, CDK-6, PCNA, WNT11, AXIN1*, and *CTNNB1* genes. Additionally, *in-silico* studies were conducted to determine the binding affinities of BMAP-27 with adenomatous polyposis coli (APC) and β-catenin proteins, one of the primary regulators of colon cancer. BMAP-27 peptide reduced colon cancer cell proliferation, upregulated tumor suppressor genes *CASPASE3, BAX, TP53, AXIN1* expression, and downregulated the expression of oncogenes *BCL-2, CDK-6, PCNA, WNT11, CTNNB1* in both the cell lines, however, in the primary colon cancer cell line the changes are found to be more significant. The molecular dynamic simulation analysis revealed substantial binding affinity of the peptide to APC and β-catenin proteins. BMAP-27 peptide significantly inhibited the proliferation and induced apoptosis in the primary colon cancer cell line than in the metastatic colon cancer cell line. *In-silico* results suggest that BMAP-27 shows a strong binding affinity with APC and β-catenin proteins, highlighting its role in inhibiting colon cancer cell proliferation.

Keywords Colon cancer · Apoptosis · BMAP-27 · Peptide therapy · Molecular docking · MD-simulation

Introduction

Peptides have emerged as a potent therapeutic candidate for several diseases, including cancer, because of their small size, facile manufacturing, easy membrane permeability, and enhanced specificity. Excessive research has supported several small therapeutic peptides, among which many are undergoing clinical trials (Bose et al. 2022; Das et al. 2022, 2023). Small peptides (20–30 amino acids) are essentially lower immunogenic and stable at room temperature than full-length proteins and antibodies (Timur and Gürsoy 2021; Karami Fath et al. 2022; Das et al. 2023). Studying the anticancer role of small peptides in several forms of cancer (Conibear et al. 2020; Bose et al. 2022; Das et al. 2022; Karami Fath et al. 2022) has now gained much attention. Colon cancer (CC) is one of the most frequent malignant cancers around the globe, and in recent years, the incidence rate has risen globally (Xi and Xu 2021; Wu and Lui 2022; Saraiva et al. 2023). Epigenetic

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and genetic alterations within a cell are intrinsic in CC, activating oncogenes and proliferative signals from the abnormal microenvironment surrounding the cell (Hossain et al. 2022). Adenomatous polyposis coli (APC), a tumor suppressor protein, is deregulated at the somatic and germline levels (Jung et al. 2020; Kawazu et al. 2022). In CC, APC gene mutations cause the APC protein to stabilize and accumulate β-catenin in the cytoplasm, and cytoplasmic β-catenin translocates to the nucleus and upregulates genes that promote uncontrolled cell growth and division (Ayala-Calvillo et al. 2017). Cellular activities, including cell adhesion, motility, differentiation, proliferation, and chromosome separation, are all influenced by the APC gene, which is found to be mutated in about 80% of colon tumors (Siraj et al. 2020; Liu et al. 2020; Wu et al. 2020; Ghasemi et al. 2021). For 80–85% of sporadic CC, changes in the APC gene are an early, if not an initiating, event, except those that display a CpG island methylator phenotype (CIMP) or hypermutable microsatellite instability (MSI) phenotype because of a lack of mismatch repair (MMR) (Fennell et al. 2020; Moshawih et al. 2022). Although there are several strategies for CC treatments, the main issues with most of these therapies include the relapse of the cancer (Noe et al. 2021; Graf et al. 2023).

The antimicrobial peptide BMAP-27 is a cathelicidin peptide characterized by an α-helical structure and a C-terminus of 27 amino acid residues amidated. The structural analysis of BMAP-27 revealed an elongated α-helix at the N-terminal region, which exhibited distinct cationic and aromatic patterns. Additionally, a central kink was seen, along with a tiny hydrophobic helix at the C-terminal end (McGwire et al. 2003; Haines et al. 2003; Lee et al. 2011; Yang et al. 2019). The peptide's cationic NH_2-terminal portion (residues 1 to 18) is predicted to create an amphipathic α-helix, followed by a hydrophobic tail (residues 19 to 27). The hydrophobic tail is discovered to be essential for the peptide to exert its cytotoxic effect. The N-terminal amphipathic helix mediates initial binding to the anionic membrane surface.

On the other hand, the C-terminal hydrophobic helix is a key structure part that allows the peptide to be rapidly and effectively inserted into the hydrophobic core of the lipid bilayer, which kills bacteria and cancer cells (Hoskin and Ramamoorthy 2008). When BMAP-27 peptide was added to different human leukemia cell lines, it was found that BMAP-27 was able to kill cancer cells, increasing the permeability of the cell membranes leading to Ca^{2+} penetration (Risso et al. 1998; Hoskin and Ramamoorthy 2008; Yang et al. 2019), subsequently causing fragmentation of the DNA, and resulting in induced apoptosis (Risso et al. 2002). The current study aimed to investigate the role of the BMAP-27 in suppressing proliferation and inducing apoptosis in colon cancer cell lines.

Materials and Methods

Chemicals

Primary (SW480) and metastatic (SW620) colon cancer cell lines were purchased from NCCS, Pune, India, where sixteen short tandem repeat (STR) profiling were done using AmpFISTR® Identifiler® Plus Amplification Kit and Applied Bio system® 3500 Genetic analyser at NCCS, Pune (Supplementary material 1). BMAP-27 peptide with the sequence (GRFKRFRKKFKKLFKKLSPVIPLLHL) was purchased from PRIVEEL PEPTIDES: CHENNAI, India, with > 90% purity.

Cell Culture Conditions

Primary (SW480) and metastatic (SW620) colon cancer cell lines were cultured in DMEM containing 10% FBS, 1% glutamine, and 1% penicillin-streptomycin at 37 °C in 5% CO_2.

Dosage Selection and Cell Proliferation Analysis

MTT Assay

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) analysis was performed for dose fixation of BMAP-27 peptide for further experiments. 2×10^3 per well of SW480 and SW620 cells were seeded in a 96-well plate and were grown under culture conditions for 24 h in standard 10% FBS in DMEM medium. After 24 h incubation, the cells were treated with various concentrations of BMAP-27 peptide (1 ng/mL, 250 ng/mL, 500 ng/mL, 750 ng/mL, 1 µg/mL) for 72 h. MTT solution (2 mg/mL) was added to the cells and kept for incubation at 37 °C for 4 h. Then 40 µl of DMSO was added to the wells, and the absorbance was taken at 546 nm using an ELISA reader (Robonik Touch Well Reader, India) (Rahimi et al. 2018). The IC_{50} value was determined through analysis of the optical density obtained from the MTT assay, using the linear equation ($y = mx + c$), where x represented the IC_{50} value of BMAP-27 peptide, and y represented the 50% viability of colon cancer cell line (Olaokun et al. 2020).

Cell Proliferation Assay

The Cell Counting Kit-8 (CCK-8, Abbkine) was used to determine cell proliferation. About 2×10^3 cells per well were seeded in a 96-well plate. After 24 h of seeding the cells, they were treated with the selected doses of BMAP-27 (500 ng/mL and 250 ng/mL) peptide and allowed to

proliferate for different intervals (24 h, 48 h, and 72 h). Absorbance was measured at 450 nm with a microplate reader (Robonik Touch Well Reader, India).

Lactate Dehydrogenase (LDH) Activity

LDH assay was performed to measure the degree of damaged cells using the EZcount™ lactate dehydrogenase cell assay kit (Cat. No: CCK036, HiMedia) as per the manufacturer's protocol.

Catalase Activity Analysis

Catalase activity was measured using a standard protocol with slight modification (Hadwan and Abed 2016).

Assessment of Cellular Senescence

β-Galactosidase Activity Assay

The β-galactosidase activity is a known biomarker of cell senescence which is measured using an EZdetect™ cell senescence detection kit (Cat. No-CCK063, HiMedia).

Gene Expression Analysis by qPCR

RNAzol (Cat. No. R4533, Sigma–Aldrich) was used to isolate RNA from the cells. NanoDrop was used to measure the concentration of total RNA in each sample at 260 and 280 nm. Eurogentec Reverse Transcriptase PCR kit (Cat. No. RT-RTCK-03, Eurogenetec) was used for the reverse transcription. Ct values were obtained for all the genes (*CASPASE*3, *BAX*, *BCL*-2, *CDK*-6, *PCNA, TP*53, *WNT*11, *AXIN*1, *CTNNB*1) with Takara Bio's RR420A Syber Green qPCR master mix (Cat. No: RR420A, Takara) and normalized with housekeeping gene *GAPDH* to generate ΔCt values. The fold change was determined by calculating the relative quantification (RQ) values ($2^{-\Delta\Delta CT}$). Further, the pre-designed primers (Sigma–Aldrich) details are given below in Table 1.

Assessment of Expression of IL-6, IFN-γ and CXCL-10, Human Cytochrome c

The evaluation of the inflammatory and apoptotic markers was performed with the help of the proteins that were isolated from the cell lysate. IL-6, IFN-γ, CXCL-10 and cytochrome c expression were studied using IL-6 ELISA kit (Cat No. KET6017, Abbkine), IFN-γ ELISA kit (Cat No. KET6011, Abbkine), CXCL-10 ELISA kit (Cat. No. KTE62958, Abbkine), and cytochrome c ELISA kit (Cat. No. KTE62179, Abbkine) according to the manufacturer's protocol.

Statistical Analysis

The statistical significance was determined with the help of the unpaired student's t-test and the GraphPad V8.4.2 software. The statistical significance of the results, (*) indicates a p value less than 0.05 ($p < 0.05$), (**) indicates a p value less than 0.01 ($p < 0.01$), (***) indicates p value less than 0.001 ($p < 0.001$) and ns = non-significant. The data

Table 1 Table representing the primer details

Gene name	Sequence (5′–3′)	Base pairs	Tm (°C)
CASPASE3	F: 5′-ATGGTTTGAGCCTGAGCAGA-3′	20	64.9
	R: 5′-GGCAGCATCATCCACACATAC-3′	21	65.3
BAX	F: 5′-GTGCCGGAACTGATCAGAAC-3′	20	64.7
	R: 5′-CCAAAGTAGGAGAGGAGGCC-3′	20	63.9
BCL2	F: 5′-GCCTTCTTTGAGTTCGGTGG-3′	20	65.9
	R: 5′-GAAATCAAACAGAGGCCGCA-3′	20	67.3
CDK-6	F: 5′-GGATATGATGTTTCAGCTTCTC-3′	22	59.2
	R: 5′-TCTGGAAACTATAGATGCGG-3′	20	59.3
PCNA	F: 5′-CTGTGTAGTAAAGATGCCTTC-3′	21	55.6
	R: 5′-TCTCTATGGTAACAGCTTCC-3′	20	56
TP53	F: 5′-ACCTATGGAAACTACTTCCTG-3′	21	56.4
	R: 5′-ACCATTGTTCAATATCGTCC-3′	20	58.7
WNT11	F: 5′-ACAACCTCAGCTACGGGCTCCT-3′	22	69.2
	R: 5′-CCCACCTTCTCATTCTTCATGC-3′	22	66.8
AXIN1	F: 5′-GTGCCCCTACCTCACATTCC-3′	20	65.6
	R: 5′-CGAACTTCTGAGGCTCCACG-3′	20	67.3
CTNNB1	F: 5′-TGAGGAGCAGCTTCAGTCCC-3′	20	67
	R: 5′-CTTGAGTAGCCATTGTCCACG-3′	21	64.5
GAPDH	F: 5′-ACAGTTGCCATGTAGACC-3′	18	55.7
	R: 5′-TTGAGCACAGGGTACTTTA-3′	19	55.8

are presented as mean ± standard error. All the experiments were performed in triplicates.

Methodology for Molecular Docking and Simulation Analysis

Using two repeating peptide sequences the structure of BMAP-27 was prepared from the Phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index), and then after generation of the structure, only one sequence is taken for molecular docking and Molecular Dynamics simulation. The PDB files of the crystal structure of adenomatous polyposis coli (APC) protein (PDB ID: 1LUJ) and β-catenin (PDB ID: 4YJL) were retrieved from the protein data bank (https://www.rcsb.org/). Both the structures of the proteins were cleaned using UCSF-chimers. The molecular docking study between BMAP-27, APC, and β-catenin protein was then performed using the patchdock web server (http://bioinfo3d.cs.tau.ac.il/PatchDock/php.php). Furthermore, to investigate the effect of BMAP-27 on APC-protein and β-catenin, we have performed MD-simulation studies using GROMACS (Version 2018.5), protein-peptide composite system using the amber99sb force field and TIP3P solvation model. A periodic boundary condition with a cubical box of 1 nm edge length was considered. Na^+ or Cl^- ions were added to neutralize the system. Energy minimization was carried out until the maximum force became < 10 $kJmol^{-1}$ nm^{-1}. Then isochoric-isothermal (NVT) followed by isobaric-isothermal (NPT) ensembles were used to equilibrate the system for 1000 ps at 300 K. For the NPT ensemble, a modified Berendsen thermostat was used. NVT and NPT equilibration 1 nm of electrostatic and van der Waals interaction cut-off was kept. Using the same electrostatic and van der Waals cut-off with the equilibrated ensembles, MD simulation for 10 ns was performed. A modified Berendsen thermostat and a Parinello–Rahman barostat were used with reference temperature and pressure at 300 K and 1 bar, respectively.

Molecular Dynamic Simulations

To realign the protein, peptide, and other molecules within the cubical box, the trjconv tool was used. Xmgrace tool was used to plot Root mean square deviation (RMSD), Radius of gyration (Rg), and solvent-accessible surface area (SASA) plots.

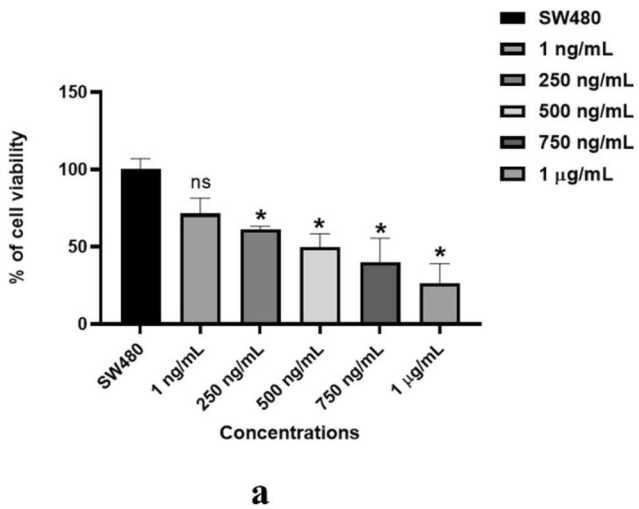
Results

MTT Assay

SW480 and SW620 cells were treated with various concentrations (1 ng/mL, 250 ng/mL, 500 ng/mL, 750 ng/mL, 1 µg/mL) of BMAP-27 peptide for 72 h. BMAP-27 was found to exhibit a strong dose as well as time-dependent reduction of cell viability. IC_{50} value of 556 ng/mL was calculated, and doses of 500 ng/mL and 250 ng/mL were selected for further study (Fig. 1).

CCK-8 Assay

The Cell Counting Kit-8 (CCK-8) was used to measure the proliferation of SW480 and SW620 cells. The BMAP-27 peptide (500 ng/mL and 250 ng/mL) showed a reduction in



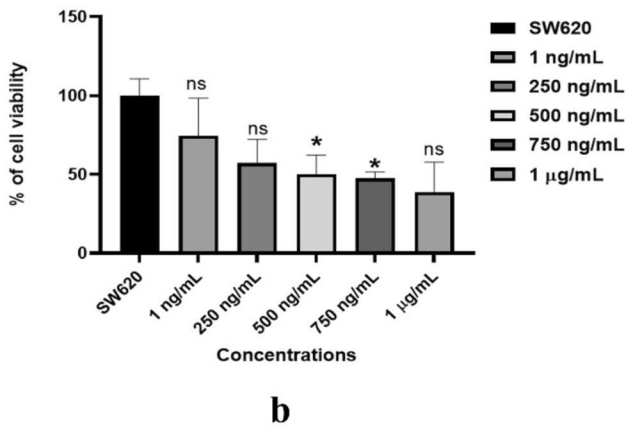


Fig. 1 Cell viability analysis of BMAP-27- treated control, primary (SW480), and metastatic (SW 620) colon cancer cells by MTT assay. **a** SW480 and BMAP-27 peptide-treated SW480 cells; **b** SW620 and BMAP-27 peptide-treated SW620 cells at 72 h. The statistical significance results represents p values $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ and ns = non significant

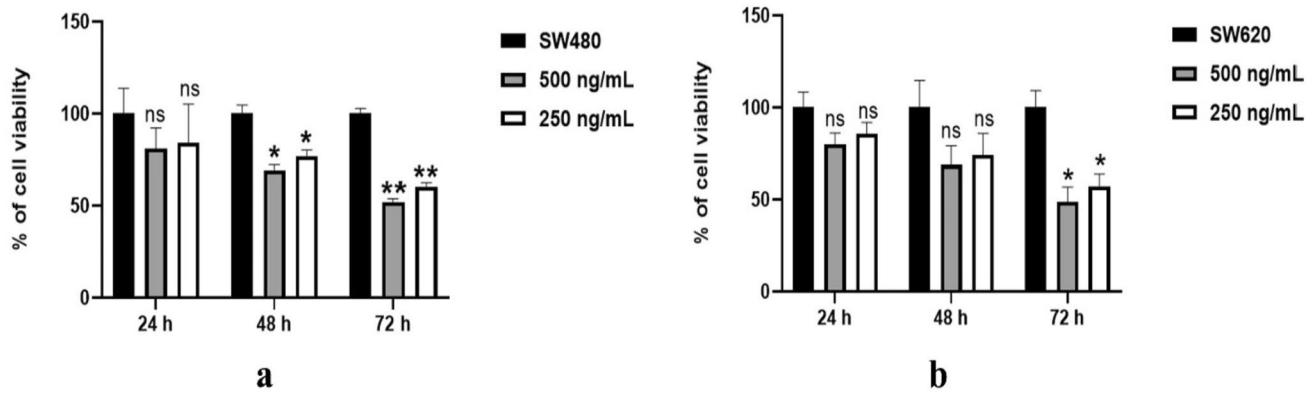


Fig. 2 Bar diagram representing the difference in proliferation between control and BMAP-27 peptide treated cells at 24, 48, and 72 h. **a** SW480 and BMAP-27 peptide-treated SW480 cells; **b** SW620 and BMAP-27 peptide-treated SW620 cells. The statistical significance results represents p values $*p<0.05$; $**p<0.01$; $***p<0.001$ and ns = non significant

cell growth compared to the untreated SW480 and SW620 cells. It was found to decrease cell survival by inhibiting growth, and statistically significant data is shown in Fig. 2a, b.

Lactate Dehydrogenase Assay

The lactate dehydrogenase (LDH) study was used to evaluate the cell-damaging potential of the BMAP-27 peptide. After 72 h of treatment with the peptide, cellular damage was seen as increased LDH release in peptide-treated SW480 cells compared to non-treated cells. However, it did not show any significance in metastatic colon cancer cell lines Fig. 3a, b.

Catalase Activity

The activity of catalase was assessed in non-treated groups as well as in the peptide-treated groups. Our findings showed that BMAP-27 peptide, at concentrations of 500 ng/mL and 250 ng/mL, respectively, produced a substantial increase in the catalase activity of primary cells, thus restoring the initial levels of catalase. However, it showed no significant difference in metastatic colon cancer cell line Fig. 4a, b.

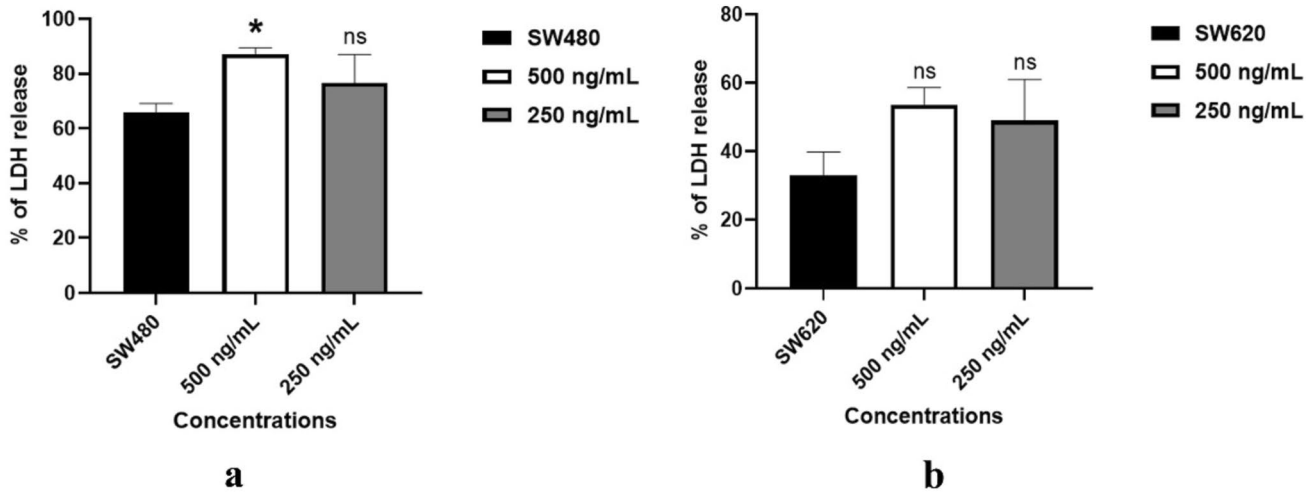


Fig. 3 Determination of cellular damage by BMAP-27 in colon cancer cells. Bar graph represents the LDH release percentage by **a** SW480 and BMAP-27 peptide-treated SW480 cells; **b** SW620 and BMAP-27 peptide-treated SW620 cells. The statistical significance results represents p values $*p<0.05$; $**p<0.01$; $***p<0.001$ and ns = non significant

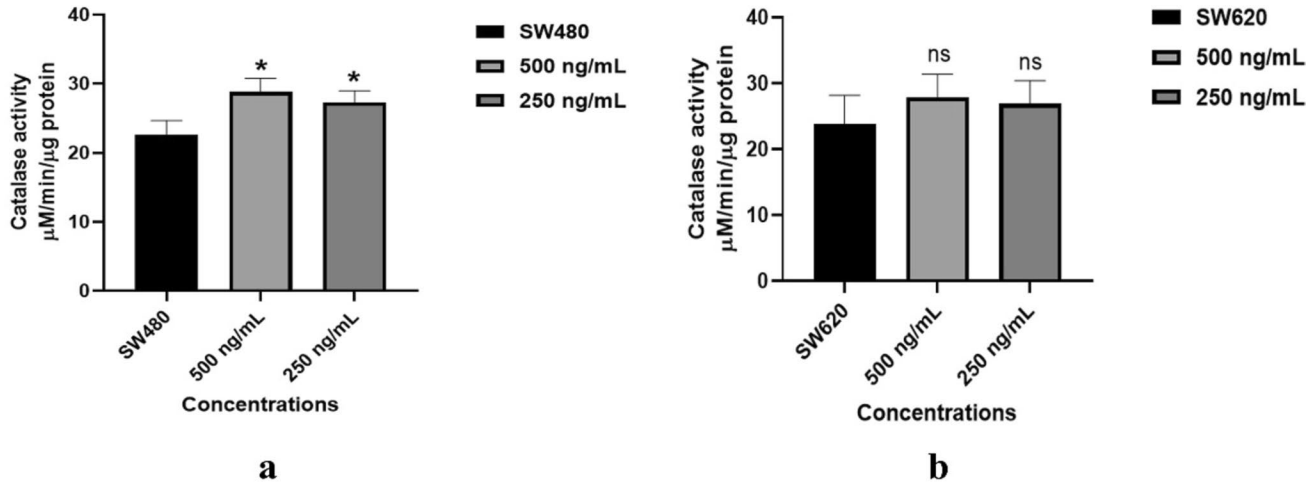


Fig. 4 Catalase activity was assessed to evaluate the impact of BMAP-27 on colon cancer cells. The figure illustrates the catalase activity observed in two scenarios: **a** SW480 cells and BMAP-27 treated SW480 cells and **b** SW620 cells and BMAP-27 treated SW620 cells. The statistical significance represents values as $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ and *ns* = non significant

Cell Senescence Assay

Senescence-Associated-β-Galactosidase (SA-β-gal) Assay

Senescence-associated-β-galactosidase enzyme expression was studied on SW480 and SW620 cells, revealing a low level of senescence. However, SW480 and SW620 cells supplemented with BMAP-27 (500 ng/mL and 250 ng/mL) peptide exhibited more significant number of blue-stained cells indicating significantly higher levels of senescence as shown in Fig. 5a, b.

Gene Expression Analysis Using qPCR

The expression of apoptotic markers *CASPASE3*, *BAX*, and *BCL-2* was analyzed by real-time PCR. In the present study, the apoptotic proteins in primary and metastatic colon cancer cell lines showed that BMAP-27 treatment for 72 h significantly regulated apoptosis in the primary colon cancer cell line compared to the metastatic colon cancer cell line. Further, BMAP-27 decreased *CDK6* and *PCNA* expression in colon cancer cell lines, indicating its cell-cycle regulatory effects in both primary and metastatic cell lines. Moreover, BMAP-27 treatment for 72 h increased *p53* expression in the primary colon cancer cell line. Subsequently, the effect of BMAP-27 was also assessed in several cancer-associated genes, where it showed a decreased expression of *WNT11* after the treatment with BMAP-27 peptide to the colon cancer cell lines. Similarly, BMAP-27 increases *AXIN1* expression, which may control *CTNNB1* and reduce the expression of β-catenin in peptide-treated colon cancer cell lines Fig. 6a, b.

Modulation of Inflammatory Markers After the Treatment of BMP 27

Our results suggested that the BMAP-27 peptide (500 ng/mL) reduced the IL-6 concentration significantly in the SW480 colon cancer cell line. However, it could not show any significant effect on metastatic colon cancer cell line. In the same line, BMAP-27 (500 ng/mL) was also able to reduce the IFN-**γ** concentration significantly in both SW480 and SW620 cells, indicating that BMAP-27 treatment might regulate the activation of immune response. Moreover, BMAP-27 significantly decreases the expression level of CXCL-10 in both primary and metastatic colon cancer cell lines, indicating the anticancer properties of BMAP-27. In the current investigation, we found that the cytochrome c level increased significantly in SW480 cells, suggesting that BMAP-27 might induce apoptosis in the colon cancer cell line. Figure 7a–h.

Molecular Docking and Molecular Simulation Study

The molecular docking study of BMAP-27 with APC protein and β-catenin was performed using patchdock server, and the results obtained from the server were tabulated in Table 2. The docking score between BMAP-27 and β-catenin is higher compared to APC protein. Also, the higher confidence score and lower RMSD of BMAP-27 prove more selective docking against β-catenin (4YJL).

Figure 8 represents the docked structure of BMAP-27 with APC protein (1LUJ) and β-catenin (4YJL). Due to the smaller binding cavity of both proteins, BMAP-27 has docked at the surface of the proteins. The minimum distance between BMAP-27 and 1LUJ was found to be 2.73 Å in the

docked structure, while that between BMAP-27 and 4YJL was found to be 2.68 Å, which suggests a good binding affinity between BMAP-27 and 4YJL.

To further investigate the interaction of BMAP-27 with the proteins (β-catenin and APC), MD simulation was performed for a period of 10 ns with the docked and undocked structures. The RMSD plots obtained from the MD-simulation are shown in Fig. 9. It is clear from Fig. 9a that the deviation of the plot is lesser after 8 ns with the BMAP-27 bind protein indicating the stabilization of the protein due to the presence of BMAP-27. While Fig. 9b indicates the stabilization of the plot after 4 ns due to the presence of BMAP-27. Furthermore, the deviation is more in the docked 1LUJ compared to 4YJL, revealing the more effectiveness of BMAP-27 against β-catenin (4YJL) compared to APC protein (1LUJ).

Figure 10 represents the Rg plot of BMAP-27 with APC protein and β-catenin. Rg-plots measure the compactness of the protein during MD simulation. The more compact the proteins more they will bind with the BMAP-27. It is clear from Fig. 10 that the proteins are more compact in the docked structures. The compactness of BMAP-27 docked 1LUJ is achieved after 8 ns, while for 4YJL, it is after 4 ns, which is consistent with the RMSD plot. The compactness is more in BMAP-27 docked 4YJL compared to 1LUJ.

To interpret the structural stability of the native structure, we have analyzed SASA. Figure 11 represents the SASA plot of BMAP-27 docked 1LUJ and 4YJL, which determines the stability and folding of proteins. SASA values for BMAP-27 docked 4YJL is lowered compared to that for 1LUJ.

In both cases, BMAP-27 docked conformers show less fluctuation (for 1LUJ-BMAP within 250 nm^2 and for 4YJL-BMAP within 190 nm^2) compared to undocked conformation, suggesting the stability of the docked conformation.

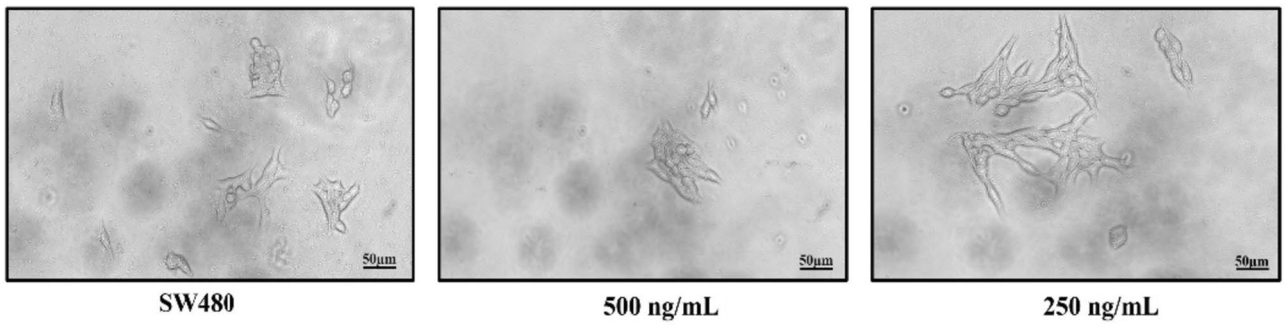
Discussion

Conventional cancer therapies are associated with multiple adverse effects due to the non-selective nature of frequently administered anticancer agents, which impacts the growth of all rapidly dividing cells rather than only targeting malignant cells. On the other hand, certain anticancer peptides appear to exhibit selectivity towards malignant cells. It is evident that additional research is required to thoroughly investigate the precise mechanism through which anticancer peptides (ACPs) interact with cancer cells. BMAP-27, which belongs to the cathelicidin family of peptides, has been found to decrease cancer cell proliferation without toxic hemolytic activity (Zanetti et al. 2002; Cragg and Pezzuto 2016; Zhang and Shay 2017; Liang et al. 2020; Lath et al. 2023). The C-terminal hydrophobic helix of the BMAP-27 peptide may be inserted into the lipid bilayer, and this contact results in membrane permeabilization and depolarization, which may be involved in its anticancer actions (Li et al. 2012; Amerikova et al. 2019; Yang et al. 2019; Yadav and Misra 2021). We observed a significant reduction in cellular proliferation in a dose and time-dependent manner (24 h, 48 h, and 72 h) in which 500 ng/mL and 250 ng/mL concentrations of the peptides showed significant results in 72 h compared to 24 and 48 h. However, it is observed from the results that BMAP-27 showed a significant impact in the primary colon cancer cell line than in the metastatic cell line.

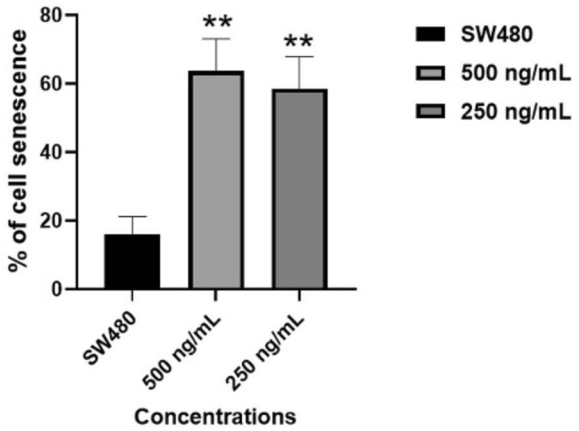
LDH levels are associated with the development of colon cancers (Tas et al. 2001; Bohers et al. 2018; Dall'Olio et al. 2022). Our results showed that BMAP-27 can induce LDH levels at a significant rate in primary colon cancer cells. However, BMAP-27 could not show a significant difference in the metastatic colon cancer cell line. An increase in LDH release can indicate cell death, particularly necrosis, in which cells often release their intracellular contents, including LDH, into the surrounding environment (Li et al. 2016).

Catalase enzymatic activity is found to be reduced in colon cancer cell lines. Furthermore, we have also observed a decreased level of catalase activity in the untreated group, which may indicate an increased susceptibility to redox imbalance and oxidative damage. It has been demonstrated to be crucial in several different cellular processes (Zińczuk et al. 2019). While hydrogen peroxide (H_2O_2) does not possess free radical properties, it has the ability to traverse the cell membrane. Hence, it is unsurprising that a reduction in the activity of catalase, the enzymes accountable for the detoxification of H_2O_2, has been found to be rational. However, our results showed a significant increase in the levels of catalase activity with the peptide-treated groups in SW480 cells. Still, they showed no substantial difference in the metastatic colon cancer cell line. Cancer cells reduce cell senescence by maintaining the telomere length, epigenetic regulation, a mutation in the tumor suppressor genes, and immune evasion (Özsoy et al. 2020; Dai et al. 2022). Senescence-associated secretory phenotype (SASP), a group of cytokines and chemokines generated by senescent cells, may cause inflammation. Thus, in SW480 and SW620 colon cancer cell lines, BMAP-27 peptide dosages significantly increased the number of blue-stained cells, indicating senescent cells compared to untreated SW480 and SW620 cells. Cells can potentially undergo senescence that may lead to apoptosis, serving as a secondary response towards cell death. Senescence can function as a defensive mechanism to inhibit the proliferation of impaired cells. However, when the extent of damage is beyond a certain threshold, cells may ultimately trigger apoptosis as a means of self-elimination.

The expression levels of *CASPASE-3, BAX*, and *BCL-2* are the potential predictors of colon cancer progression. Elevated expression of *BCL-2* or decreased expression of *BAX* is associated with a more unfavorable prognosis



SW480 500 ng/mL 250 ng/mL



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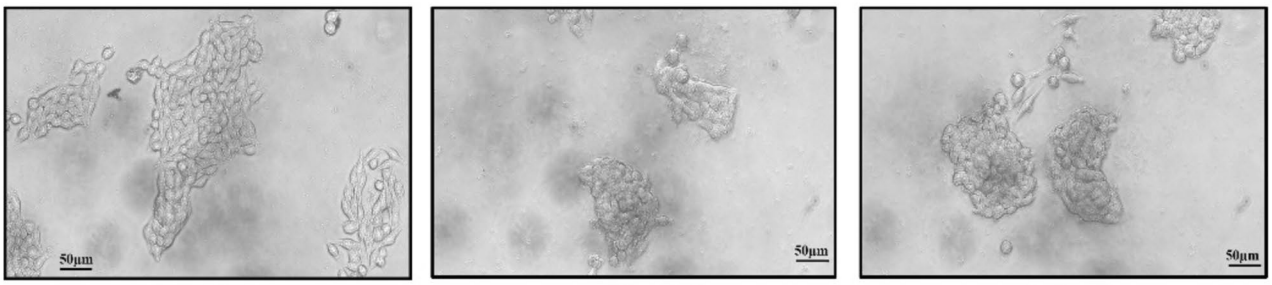
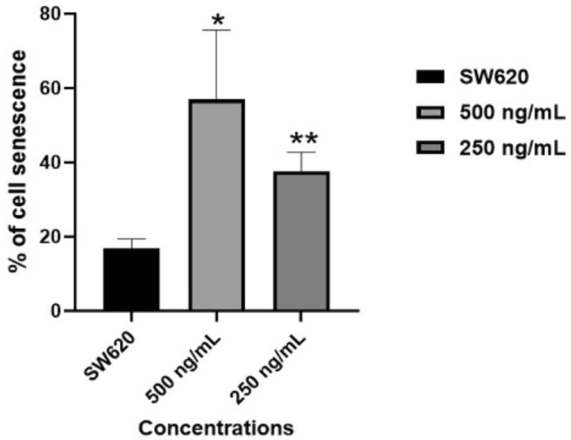


SW620 500 ng/mL 250 ng/mL



b

◂**Fig. 5** β-Gal cell senescence analysis in control and BMAP-27 treated cells. **a** SW480 and BMAP-27 peptide-treated SW480 cells; **b** SW620 and BMAP-27 peptide-treated SW620 cells. The statistical significance represents values as $*p<0.05$; $**p<0.01$; $***p<0.001$ and *ns* = non significant

(Scopa et al. 2001; Khodapasand et al. 2015). The expression of pre and anti-apoptotic genes on primary and metastatic colon cancer cell lines indicates that the administration of BMAP-27 for 72 h induces apoptosis. The genes *CDK6, PCNA*, and *WNT11* showed a decrease in expression. Previous research has demonstrated that *TP53* mutations were consistently present in 80% of individuals with advanced cervical cancer and metastases (Li 2015; Zhou et al. 2018; Nakayama and Oshima 2019; Gorroño-Etxebarria et al. 2019; Yousuf et al. 2020). In the present study, we also found that colon cancer cell lines showed reduced levels of *p53* expression. Still, after the administration of the BMAP-27 peptide, a significant upregulation of *p53* expression in the primary colon cancer cell line was observed. The development of colon cancer is closely linked to the inactivation of the *AXIN1* gene (Sanson et al. 2023).

In some cases of colon cancer, mutations in the *AXIN1* gene have been found to enhance the different proteins involved in the Wnt/β-catenin signaling pathway. Dysfunction of *AXIN1* leads to the nuclear accumulation and stability of *β-catenin*, resulting in the upregulation of target genes like c-myc and cyclin D1 that facilitates cell proliferation, survival, and development of cancer (Nakamura et al. 1998). Our findings demonstrate that the administration of

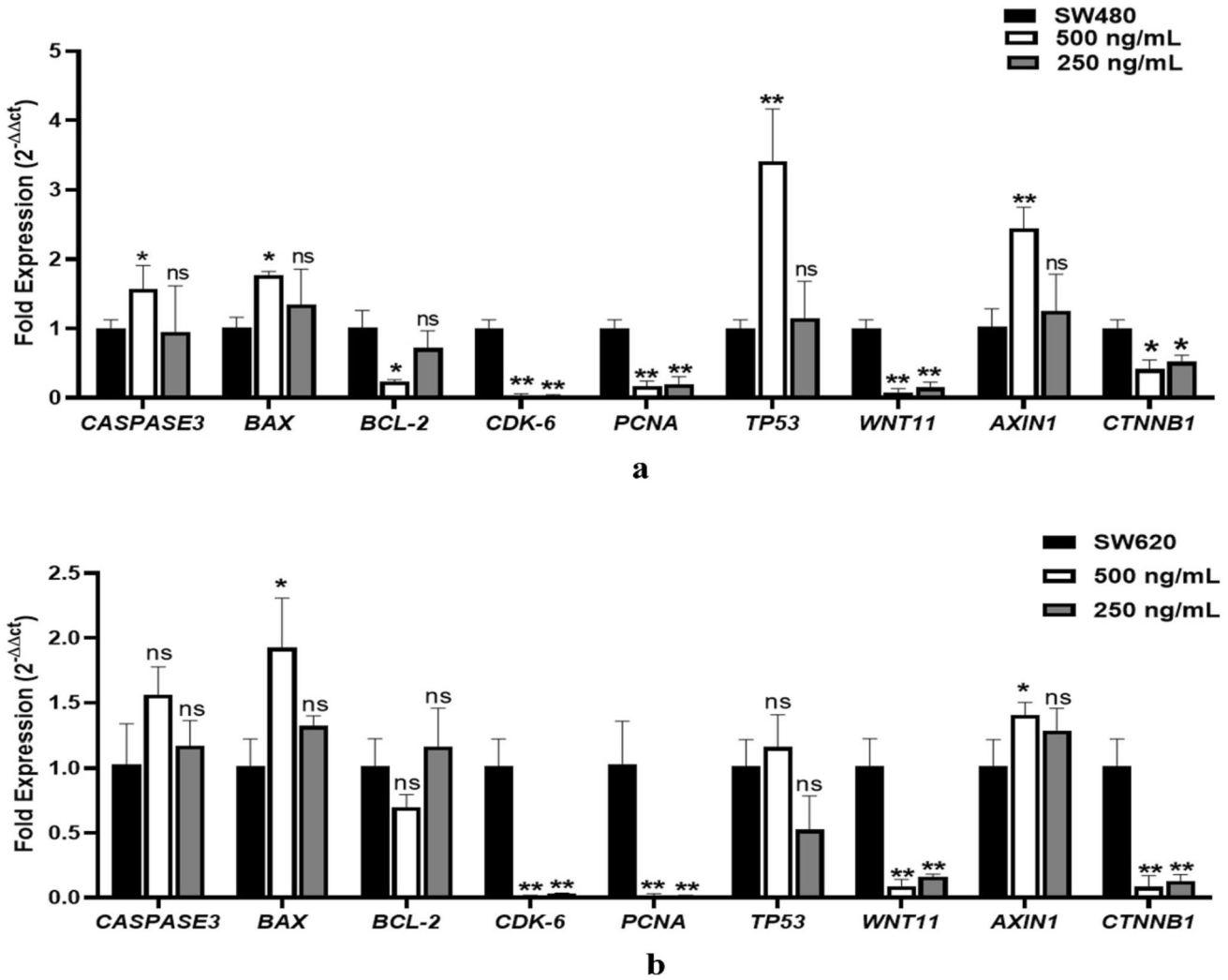
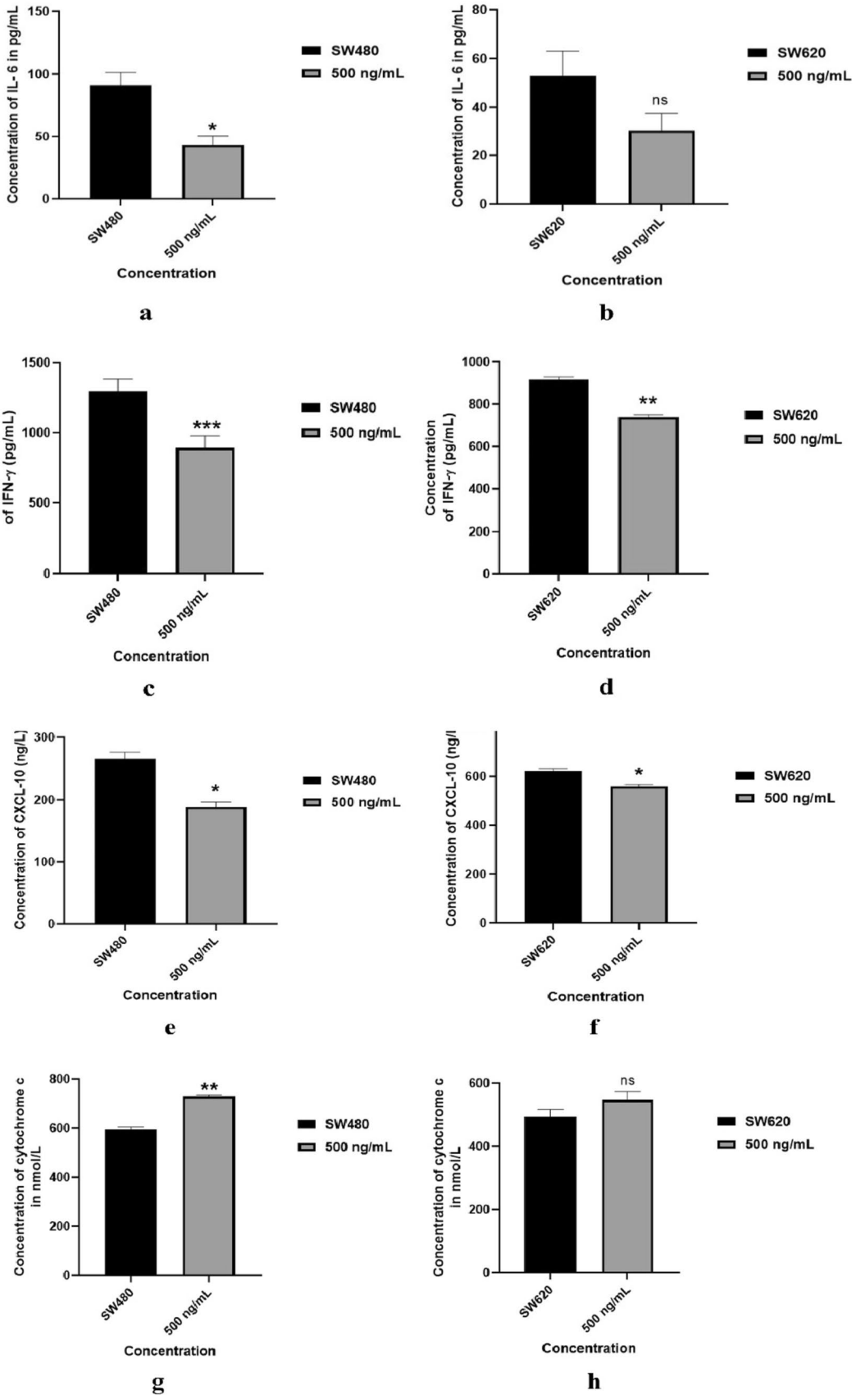


Fig. 6 Expression profile of pro-apoptotic marker *CASPASE3* and *BAX*, anti-apoptotic marker *BCL-2*, cell proliferation markers *CDK-6* and *PCNA*, tumour suppressor marker *TP53*, cancer-specific markers *WNT11*, *AXIN1*, and *CTNNB1* in **a** SW480 and BMAP-27 peptide-treated SW480 cells; **b** SW620 and BMAP-27 peptide treated SW620 cells. The statistical significance represents values as $*p<0.05$; $**p<0.01$; $***p<0.001$ and *ns* = non significance


Concentration of IL- 6 in pg/mL
150
100
50
0
*
SW480
500 ng/mL
Concentration
SW480
500 ng/mL
a
Concentration of IL- 6 in pg/mL
80
60
40
20
0
ns
SW620
500 ng/mL
Concentration
SW620
500 ng/mL
b
Concentration of IFN-γ (pg/mL)
1500
1000
500
0
***
SW480
500 ng/mL
Concentration
SW480
500 ng/mL
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Concentration of IFN-γ (pg/mL)
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SW620
500 ng/mL
Concentration
SW620
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d
Concentration of CXCL-10 (ng/L)
300
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e
Concentration of CXCL-10 (ng/l
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SW620
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Concentration
SW620
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f
Concentration of cytochrome c in nmol/L
800
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0
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SW480
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Concentration of cytochrome c in nmol/L
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◂**Fig. 7** Effect of BMAP-27 on inflammatory and apoptotic markers expression in control, primary, and metastatic colon cancer cells. Bar diagram representing the concentration of **a**, **b** Expression of IL-6, **c**, **d** Expression of IFN-γ, **e**, **f** Expression of CXCL-10, **g**, **h** Expression of cytochrome c in SW480, SW620, and BMAP-27 peptide-treated SW480 and SW620 cells. The statistical significance represents values as $*p<0.05$; $**p<0.01$; $***p<0.001$ and *ns* = non significant

BMAP-27 peptide treatment leads to a significant upregulation of *AXIN1* expression and downregulating *β-catenin* expression.

Table 2 Molecular docking results between BMAP-27 of APC-protein and β-catenin

System	Docking score	Confidence score	BMAP RMSD (Å)
1LUJ-BMAP-27	− 249.43	0.8796	29.79
4YJL-BMAP-27	− 284.80	0.9368	22.56

IL-6 promotes various malignancies, including CC (Lin et al. 2013). In the same line with other studies, colon cancer cell lines had an increased level of IL-6. However, the primary colon cancer cell line treated with BMAP-27 peptide showed significantly lower levels of IL-6. The decrease in IL-6 expression may suggest that the peptide has an anti-inflammatory effect. The relationship between inflammation and cancer growth is highly related, with the pro-inflammatory cytokine IL-6 significantly promoting inflammation. The reduction of IL-6 levels may indicate the anti-inflammatory potential of BMAP-27.

Moreover, the BMAP-27 treatment decreased the levels of inflammatory cytokine IFN-γ in SW480 and SW620 cells, confirming its anti-inflammatory potential. CXCL10, which promotes tumor growth, migration, and invasion in colon cancer (Wang et al. 2021), was observed to be decreased after the peptide treatment in primary and metastatic colon cancer cell lines, suggesting that BMAP-27 may have anticancer activities.

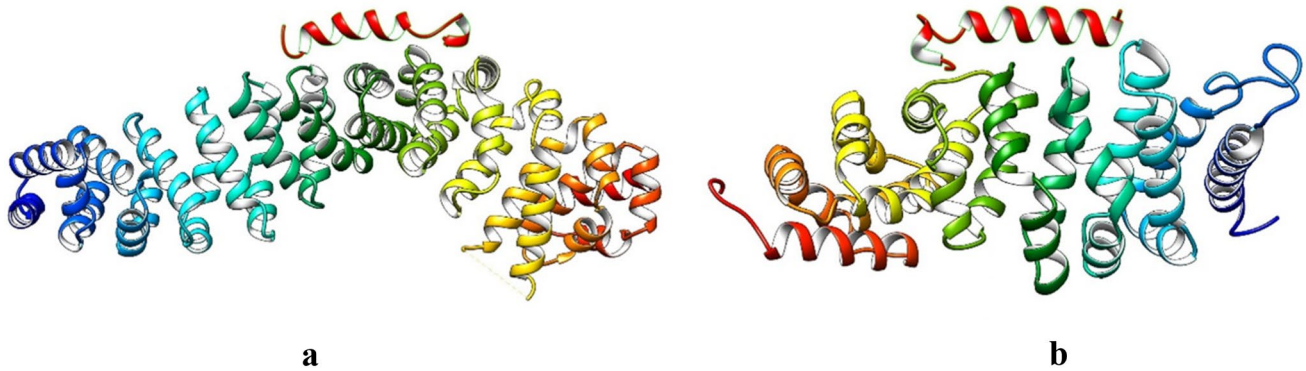

Fig. 8 Docked structure of **a** 1LUJ and BMAP-27 and **b** 4YJL and BMAP-27

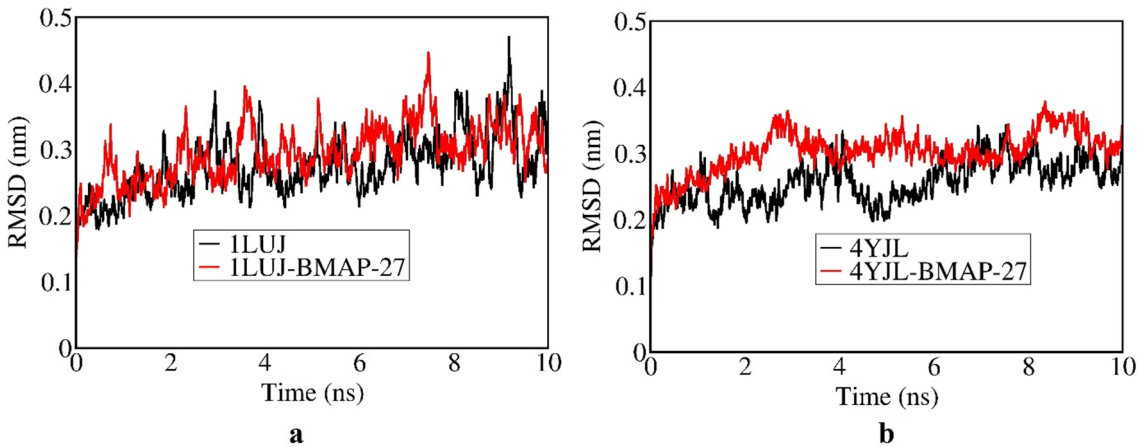

Fig. 9 RMSD plot of **a** 1LUJ and BMAP-27 and **b** 4YJL and BMAP-27

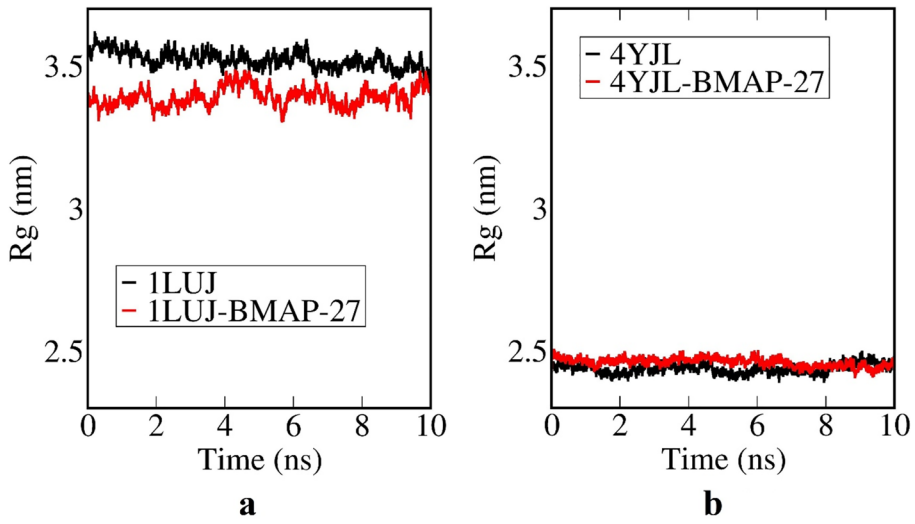

Fig. 10 Radius of gyration plot of **a** 1LUJ and BMAP-27 and **b** 4YJL and BMAP-27

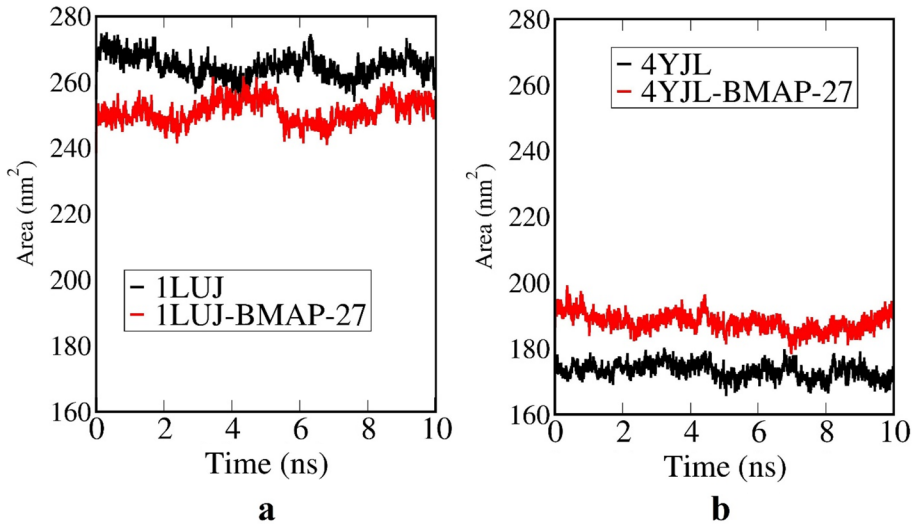

Fig. 11 Solvent-accessible surface area of **a** 1LUJ and BMAP-27 and **b** 4YJL and BMAP-27

Furthermore, reduced cytochrome c expression or subcellular localization has been linked to worse cancer outcomes, including advanced tumor stage, higher metastatic risk, and worse survival rates (Robichaux et al. 2023). In the present study, SW480 and SW620 cells treated with BMAP-27 peptide significantly increased the cytochrome c expression. An increase in cytochrome c expression in peptide-treated colon cancer cell lines may be correlated with the induction of apoptosis. The mitochondrial dysfunction may increase cytochrome c expression as the cell attempts to respond to the stress, causing cytochrome c to be released into the cytoplasm, which may trigger the apoptotic cascade.

The in-silico analyses on the effect of BMAP-27 peptide on APC and β-catenin proteins were studied through molecular docking studies followed by molecular dynamics simulation. The results indicated significant binding between BMAP-27 and the selected proteins with a high

binding affinity, revealing strong binding interactions. The molecular dynamics simulation conducted throughout 10 ns in a water-based environment provided additional evidence regarding the impact of BMAP-27 on the structural arrangement of both β-catenin and APC proteins. The RMSD plot showed less fluctuation in the case of docked proteins during MD simulation, indicating their stability and compactness. The radius of the gyration plot also demonstrated increased compactness of both proteins after binding with BMAP-27. Additionally, the solvent-accessible surface area plot showed more scattered conformation in the docked state, suggesting increased stability and access to solvent molecules like water.

Conclusions

BMAP-27 peptide demonstrated the ability to induce cell death, inhibit cell proliferation, and regulate cancer-specific gene regulation. Moreover, the present and initial data found that BMAP-27 activity was more in the primary colon cancer cell line (SW480) than in the metastatic colon cancer cell line (SW620). The molecular docking and MD simulations further revealed the interactions between BMAP-27 and its target proteins, APC and β-catenin, providing valuable insights into the mechanisms underlying the anticancer properties of BMP 27. Further research on different colon cancer cell lines and in vivo studies, including zebrafish and colon cancer mice models, will be necessary to validate the anti-colon cancer potential of BMAP-27.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10989-023-10572-9.

Acknowledgements The authors are thankful to Chettinad Academy of Research and Education (CARE), Chettinad Hospital and Research Institute (CHRI), for providing the infrastructural, financial support and fellowship to AD and InfoGenom R&D Laboratories of Cukurova University Technopolis also for financial support to complete this work.

Author Contributions The study was designed by SP, ADR, AB, AB; the manuscript was written by AD, DD, NB. All the experiments was done by AD, NB, DD, reviewed and edited by AB, AB, SA, NB, ADR and SP.

Funding Open access funding provided by University of Oslo (incl Oslo University Hospital). This study was supported by grants from Chettinad Academy of Research and Education, CARE, India, and InfoGenom R&D Laboratories of Cukurova University Technopolis (Project Reference Number: 008/12–22). The funding source had no role in the experiment and analysis of the data or in providing any materials like peptides used in this study.

Declarations

Competing Interests The authors declared no conflicts of interest, financial or otherwise.

References

Amerikova M, Pencheva El-Tibi I, Maslarska V et al (2019) Antimicrobial activity, mechanism of action, and methods for stabilisation of defensins as new therapeutic agents. Biotechnol Biotechnol Equip 33:671–682. https://doi.org/10.1080/13102818.2019.1611385

Ayala–Calvillo E, Mojica–Vï¿½zquez L, Garcï¿½a–Carrancï¿½ A, Gonzï¿½lez–Maya L (2017) Wnt/β–catenin pathway activation and silencing of the APC gene in HPV–positive human cervical cancer–derived cells. Mol Med Rep. https://doi.org/10.3892/mmr.2017.7853

Bohers E, Viailly P-J, Becker S et al (2018) Non-invasive monitoring of diffuse large B-cell lymphoma by cell-free DNA high-throughput targeted sequencing: analysis of a prospective cohort. Blood Cancer J 8:74. https://doi.org/10.1038/s41408-018-0111-6

Bose D, Roy L, Chatterjee S (2022) Peptide therapeutics in the management of metastatic cancers. RSC Adv 12:21353–21373. https://doi.org/10.1039/D2RA02062A

Conibear AC, Schmid A, Kamalov M et al (2020) Recent advances in peptide-based approaches for cancer treatment. Curr Med Chem 27:1174–1205. https://doi.org/10.2174/0929867325666171123204851

Cragg GM, Pezzuto JM (2016) Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. Med Princ Pract 25:41–59. https://doi.org/10.1159/000443404

Dai L, Wang X, Bai T et al (2022) Identification of a novel cellular senescence-related signature for the prediction of prognosis and immunotherapy response in colon cancer. Front Genet. https://doi.org/10.3389/fgene.2022.961554

Dall'Olio FG, Marabelle A, Caramella C et al (2022) Tumour burden and efficacy of immune-checkpoint inhibitors. Nat Rev Clin Oncol 19:75–90. https://doi.org/10.1038/s41571-021-00564-3

Das A, Deka D, Banerjee A et al (2022) A concise review on the role of natural and synthetically derived peptides in colorectal cancer. Curr Top Med Chem 22:2571–2588. https://doi.org/10.2174/1568026622666220516105049

Das A, Adhikari S, Deka D et al (2023) An updated review on recent advances in the usage of novel therapeutic peptides for breast cancer treatment. Int J Pept Res Ther 29:32. https://doi.org/10.1007/s10989-023-10503-8

Fennell LJ, Kane A, Liu C et al (2020) APC mutation marks an aggressive subtype of BRAF mutant colorectal cancers. Cancers (Basel) 12:1171. https://doi.org/10.3390/cancers12051171

Ghasemi T, Khalaj-Kondori M, Hosseinpour feizi MA, Asadi P (2021) Aberrant expression of lncRNAs SNHG6, TRPM2-AS1, MIR4435-2HG, and hypomethylation of TRPM2-AS1 promoter in colorectal cancer. Cell Biol Int 45:2464–2478. https://doi.org/10.1002/cbin.11692

Gorroño-Etxebarria I, Aguirre U, Sanchez S et al (2019) Wnt-11 as a potential prognostic biomarker and therapeutic target in colorectal cancer. Cancers (Basel) 11:908. https://doi.org/10.3390/cancers11070908

Graf MR, Apte S, Terzo E et al (2023) Novel read through agent: ZKN-0013 demonstrates efficacy in APCmin model of familial adenomatous polyposis. J Mol Med 101:375–385. https://doi.org/10.1007/s00109-023-02291-x

Hadwan MH, Abed HN (2016) Data supporting the spectrophotometric method for the estimation of catalase activity. Data Br 6:194–199. https://doi.org/10.1016/j.dib.2015.12.012

Haines LR, Hancock REW, Pearson TW (2003) Cationic antimicrobial peptide killing of african trypanosomes and *Sodalis glossinidius*, a bacterial symbiont of the insect vector of sleeping sickness. Vector-Borne Zoonotic Dis 3:175–186. https://doi.org/10.1089/153036603322662165

Hoskin DW, Ramamoorthy A (2008) Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta-Biomembr 1778:357–375. https://doi.org/10.1016/j.bbamem.2007.11.008

Hossain MS, Karuniawati H, Jairoun AA et al (2022) Colorectal cancer: a review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. Cancers (Basel) 14:1732. https://doi.org/10.3390/cancers14071732

Jung G, Hernández-Illán E, Moreira L et al (2020) Epigenetics of colorectal cancer: biomarker and therapeutic potential. Nat Rev Gastroenterol Hepatol 17:111–130. https://doi.org/10.1038/s41575-019-0230-y

Karami Fath M, Babakhaniyan K, Zokaei M et al (2022) Anticancer peptide-based therapeutic strategies in solid tumors. Cell Mol Biol Lett 27:33. https://doi.org/10.1186/s11658-022-00332-w

Kawazu M, Ueno T, Saeki K et al (2022) HLA class I analysis provides insight into the genetic and epigenetic background of immune evasion in colorectal cancer with high microsatellite instability. Gastroenterology 162:799–812. https://doi.org/10.1053/j.gastro.2021.10.010

Khodapasand E, Jafarzadeh N, Farrokhi F et al (2015) Is Bax/Bcl-2 ratio considered as a prognostic marker with age and tumor location in colorectal cancer? Iran Biomed J 19:69–75. https://doi.org/10.6091/ibj.1366.2015

Lath A, Santal AR, Kaur N et al (2023) Anticancer peptides: their current trends in the development of peptide-based therapy and anti-tumor drugs. Biotechnol Genet Eng Rev 39:45–84. https://doi.org/10.1080/02648725.2022.2082157

Lee EK, Kim Y-C, Nan YH, Shin SY (2011) Cell selectivity, mechanism of action and LPS-neutralizing activity of bovine myeloid antimicrobial peptide-18 (BMAP-18) and its analogs. Peptides 32:1123–1130. https://doi.org/10.1016/j.peptides.2011.03.024

Li X-L (2015) p53 mutations in colorectal cancer- molecular pathogenesis and pharmacological reactivation. World J Gastroenterol 21:84. https://doi.org/10.3748/wjg.v21.i1.84

Li S-A, Lee W-H, Zhang Y (2012) Efficacy of OH-CATH30 and its analogs against drug-resistant bacteria in vitro and in mouse models. Antimicrob Agents Chemother 56:3309–3317. https://doi.org/10.1128/AAC.06304-11

Li G, Wang Z, Xu J et al (2016) The prognostic value of lactate dehydrogenase levels in colorectal cancer: a meta-analysis. BMC Cancer 16:249. https://doi.org/10.1186/s12885-016-2276-3

Liang S, Mao Y, Liao M et al (2020) Gut microbiome associated with APC gene mutation in patients with intestinal adenomatous polyps. Int J Biol Sci 16:135–146. https://doi.org/10.7150/ijbs.37399

Lin J-T, Wang J-Y, Chen M-K et al (2013) Colon cancer mesenchymal stem cells modulate the tumorigenicity of colon cancer through interleukin 6. Exp Cell Res 319:2216–2229. https://doi.org/10.1016/j.yexcr.2013.06.003

Liu P, Liang B, Liu M et al (2020) Oncogenic mutations in Armadillo repeats 5 and 6 of β-Catenin reduce binding to APC, increasing signaling and transcription of target genes. Gastroenterology 158:1029–1043e10. https://doi.org/10.1053/j.gastro.2019.11.302

McGwire BS, Olson CL, Tack BF, Engman DM (2003) Killing of african trypanosomes by antimicrobial peptides. J Infect Dis 188:146–152. https://doi.org/10.1086/375747

Moshawih S, Lim AF, Ardianto C et al (2022) Target-based small molecule drug discovery for colorectal cancer: a review of molecular pathways and in silico studies. Biomolecules 12:878. https://doi.org/10.3390/biom12070878

Nakamura T, Hamada F, Ishidate T et al (1998) Axin, an inhibitor of the wnt signalling pathway, interacts with β-catenin, GSK-3β and APC and reduces the β-catenin level. Genes Cells 3:395–403. https://doi.org/10.1046/j.1365-2443.1998.00198.x

Nakayama M, Oshima M (2019) Mutant p53 in colon cancer. J Mol Cell Biol 11:267–276. https://doi.org/10.1093/jmcb/mjy075

Noe O, Filipiak L, Royfman R et al (2021) Adenomatous polyposis coli in cancer and therapeutic implications. Oncol Rev. https://doi.org/10.4081/oncol.2021.534

Olaokun OO, Alaba AE, Ligege K, Mkolo NM (2020) Phytochemical content, antidiabetes, anti-inflammatory antioxidant and cytotoxic activity of leaf extracts of *Elephantorrhiza elephantina* (Burch.) Skeels. South Afr J Bot 128:319–325. https://doi.org/10.1016/j.sajb.2019.11.030

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

Rahimi A, Amiri I, Roushandeh AM et al (2018) Sublethal concentration of H_2O_2 enhances the protective effect of mesenchymal stem cells in rat model of spinal cord injury. Biotechnol Lett 40:609–615. https://doi.org/10.1007/s10529-017-2499-7

Risso A, Zanetti M, Gennaro R (1998) Cytotoxicity and apoptosis mediated by two peptides of Innate Immunity. Cell Immunol 189:107–115. https://doi.org/10.1006/cimm.1998.1358

Risso A, Braidot E, Sordano MC et al (2002) BMAP-28, an antibiotic peptide of Innate Immunity, induces cell death through opening of the mitochondrial permeability transition pore. Mol Cell Biol 22:1926–1935. https://doi.org/10.1128/MCB.22.6.1926-1935.2002

Robichaux DJ, Harata M, Murphy E, Karch J (2023) Mitochondrial permeability transition pore-dependent necrosis. J Mol Cell Cardiol 174:47–55. https://doi.org/10.1016/j.yjmcc.2022.11.003

Sanson R, Lazzara SL, Cune D et al (2023) Axin1 protects colon carcinogenesis by an immune-mediated effect. Cell Mol Gastroenterol Hepatol 15:689–715. https://doi.org/10.1016/j.jcmgh.2022.10.017

Saraiva MR, Rosa I, Claro I (2023) Early-onset colorectal cancer: a review of current knowledge. World J Gastroenterol 29:1289–1303. https://doi.org/10.3748/wjg.v29.i8.1289

Scopa CD, Vagianos C, Kardamakis D et al (2001) bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with rectal Cancer. Appl Immunohistochem Mol Morphol 9:329–334. https://doi.org/10.1097/00129039-200112000-00007

Siraj AK, Kumar Parvathareddy S, Pratheeshkumar P et al (2020) APC truncating mutations in middle eastern population: tankyrase inhibitor is an effective strategy to sensitize APC mutant CRC to 5-FU chemotherapy. Biomed Pharmacother 121:109572. https://doi.org/10.1016/j.biopha.2019.109572

Tas F, Aykan F, Alici S et al (2001) Prognostic factors in pancreatic carcinoma. Am J Clin Oncol 24:547–550. https://doi.org/10.1097/00000421-200112000-00003

Timur SS, Gürsoy RN (2021) The role of peptide-based therapeutics in oncotherapy. J Drug Target 29:1048–1062. https://doi.org/10.1080/1061186X.2021.1906884

Wang Z, Ao X, Shen Z et al (2021) TNF-α augments CXCL10/CXCR3 axis activity to induce epithelial-mesenchymal transition in colon cancer cell. Int J Biol Sci 17:2683–2702. https://doi.org/10.7150/ijbs.61350

Wu CW-K, Lui RN (2022) Early-onset colorectal cancer: current insights and future directions. World J Gastrointest Oncol 14:230–241. https://doi.org/10.4251/wjgo.v14.i1.230

Wu Q, Wang D, Zhang Z et al (2020) DEFB4A is a potential prognostic biomarker for colorectal cancer. Oncol Lett 20:1–1. https://doi.org/10.3892/ol.2020.11975

Xi Y, Xu P (2021) Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol 14:101174. https://doi.org/10.1016/j.tranon.2021.101174

Yadav V, Misra R (2021) A review emphasizing on utility of heptad repeat sequence as a tool to design pharmacologically safe peptide-based antibiotics. Biochimie 191:126–139. https://doi.org/10.1016/j.biochi.2021.09.001

Yang S, Lee CW, Kim HJ et al (2019) Structural analysis and mode of action of BMAP-27, a cathelicidin-derived antimicrobial peptide. Peptides 118:170106. https://doi.org/10.1016/j.peptides.2019.170106

Yousuf M, Khan P, Shamsi A et al (2020) Inhibiting CDK6 activity by quercetin is an attractive strategy for cancer therapy. ACS Omega 5:27480–27491. https://doi.org/10.1021/acsomega.0c03975

Zanetti M, Gennaro R, Skerlavaj B et al (2002) Cathelicidin peptides as candidates for a novel class of antimicrobials. Curr Pharm Des 8:779–793. https://doi.org/10.2174/1381612023395457

Zhang L, Shay JW (2017) Multiple roles of APC and its therapeutic implications in colorectal cancer. JNCI J Natl Cancer Inst. https://doi.org/10.1093/jnci/djw332

Zhou H, Huang T, Xiong Y et al (2018) The prognostic value of proliferating cell nuclear antigen expression in colorectal cancer. Med (Baltim) 97:e13752. https://doi.org/10.1097/MD.0000000000013752

Zińczuk M et al (2019) Antioxidant barrier, redox status, and oxidative damage to biomolecules in patients with colorectal cancer. Can malondialdehyde and catalase be markers of colorectal cancer advancement? Biomolecules 9:637. https://doi.org/10.3390/biom9100637