5. Discussion

CRC is the most common form of lower gastrointestinal cancer \(^{227}\). It is a heterogeneous disease, the molecular and genetic features of the tumor determine the prognosis and response to (targeted) treatment \(^{228}\). Among genetic factors, the adenomatous polyposis coli (APC) gene plays a role in cancer development. According to statistical data, the incidence of colon cancer is increasing in developed and developing countries. The development of CRC is often characterized at an early stage by hyperproliferation of the colonic epithelium leading to the formation of adenomas. This is mainly a consequence of deregulated cell cycle control and/or suppressed apoptosis as usually observed in colon cancers \(^{229,230}\). In addition, several studies have reported that the loss of control of apoptosis results in cancer initiation, progression and thus many new treatment strategies targeting apoptosis are feasible that may be used in the treatment of various types of cancers \(^{231–234}\).

Many studies have been working to develop conventional or complementary therapies for treating this cancer, including surgery, chemotherapy, radiation, nutritional therapy and physical rehabilitation. In spite of many therapies, successful treatment has not yet been discovered and interrupted apoptosis occurs with drug resistance of cancer cells, which has become a blocking factor \(^{86}\).

Conventional anticancer chemotherapies that are based on alkylating agents, antimetabolites are heterogeneous in their mode of action and most of them also act against healthy cells and tissues, consequently causing deleterious side effects \(^{122}\). Moreover, conventional chemotherapies need to penetrate target cells but cancer cells can develop resistance by cellular changes that include increased expression of multidrug resistant proteins, altered interactions between the drug and its target, an increased ability to repair DNA damage and defects in the cellular machinery that mediate apoptosis \(^{88}\).
AMPs, which are effective molecules of innate immunity, have recently been shown to exhibit anticancer activity, they have gained much attention as potential anticancer agents\textsuperscript{(235)}. AMPs with cancer selective toxicity have become a potential source of alternative chemotherapeutic agents that overcome the limitations of current drugs. AMPs have been commonly considered to cause cancer cells to undergo rapid cell death through a direct cell membrane-damaging effect, but some AMPs can trigger cancer cell death through non-membranolytic intracellular actions\textsuperscript{(236,237)}.

Magainin II with antimicrobial activity has widely been defined as crucial components of host defense, which appear to act by disrupting the plasma membrane leading to the lysis of the cell. Irrespective of their antimicrobial function, magainin II constitute potential candidates for clinical applications against different infectious agents\textsuperscript{(238)}. Studies have demonstrated that magainin II represent an appropriate antimicrobial peptide in the treatment of sexually transmitted infections promoted by human immunodeficiency virus or herpes simplex virus\textsuperscript{(239)}. On the basis of this important preliminary consideration, magainin II provides promising antineoplastic activity, which renders it potentially useful as an agent for colorectal tumor therapy. In the present study, we have explored the cytotoxic and apoptotic potential of magainin II on mutant p53 and wild p53 colon cancer cell lines.

5.1 Cytotoxicity assay

In this study, cytotoxicity of magainin II on mutant p53 (HT-29, COLO 205, COLO 320 DM and HCT15), wild p53 (HCT116) colon cancer cell lines and normal cell line (Vero and MDCK) were evaluated and compared with 5-FU by MTT assay.

The IC\textsubscript{50} concentration of 5-FU were found to be 32.8 µM, 26.52 µM, 24.6 µM, 75.21 µM, 18.94 µM for HT-29, COLO 205, COLO 320 DM, HCT-15 and HCT 116 respectively.
It has been documented that cytotoxicity of 5-FU against human adenocarcinoma HT-29 cell line with IC$_{50}$ concentration of 20±3 µM. Boersma et al., explored the effect of 5-FU at 28µM and 23 µM on the viability of COLO 205 and COLO 320 DM colon cancer cell lines$^{(240)}$. Ansil et al., observed cytotoxicity effect of 5FU on HCT-15 cell line was 50µM$^{(241)}$. Chen et al., observed that 5-FU reduced viability to 56% at 48 µM. on HCT116 cell line$^{(242)}$.

The IC$_{50}$ concentration of magainin II on colon cancer cells were found to be 293.9 nM, 147.9 nM, 89.34 nM, 386.2 nM and 76.72 nM respectively. Magainin II and 5-FU both inhibit the viability of colon cancer cells for 24h in a dose-dependent fashion. The present result showed that magainin II strongly reduces the viability of colon cancer cell lines.

In agreement with our findings, the studies by Anghel et al., explored the cytotoxicity of magainin II with average IC$_{50}$ concentration 120 µM against MDA-MB-231 and M14K tumour Cell lines for MTT assay$^{(182)}$. Lehmann et al., demonstrated the cytotoxicity of magainin II against RT4, 647V, and 486P bladder cancer with average IC$_{50}$ concentration of 75.2 µM for the BrdU assay$^{(186)}$. Soballe et al. and Baker et al. also showed significant antitumor activity of magainin II against human melanomas and lung cancer cells$^{(190,191)}$. Baker et al., showed magainin analogues has in vivo anticancer ability against four murine peritoneal ascites tumours, two murine leukemias cells (L1210 and P388), an ascite sarcoma cell (S180) as well as a murine ovarian teratoma (SOT)$^{(191)}$.

In our study, a significant increase in the level of LDH release was observed in IC$_{50}$ of magainin II treated colon cancer cell lines. This might be due to increased permeability of plasma membrane coupled with excessive leakage of LDH from the cells into the medium. In addition according to the neutral red assay, reduction of cell viability in colon cancer cell lines appear to be affected following incubation with IC$_{50}$ of magainin II, indicating that magainin II altered the endosomal and lysosomal compartments of these cell lines. In
concordance with our results Ohsaki et al., and Cruciani et al., showed that growth of small-cell lung cancer cells was inhibited at concentrations 10 to 20 times lower than their normal counterparts \(^{(192,102)}\). Lu et al., demonstrated that PFR peptide produced a dose and time-dependent increase of LDH release in MEL cells \(^{(123)}\).

### 5.2 Selective toxicity of magainin II on normal cell lines

In the present study Vero cell lines and MDCK cell lines showed significantly higher IC\(_{50}\) values in MTT assays as compared to colon cancer cell lines. The IC\(_{50}\) of magainin II for these normal cells was within the range of 800–1000 \(\mu\)M. This suggests that magainin II showed a selective inhibitory effect on colon cancer cell viability versus normal cells. The study by Lehmann et al., showed that the normal murine and human fibroblast cell lines were not affected by magainin II and their IC\(_{50}\) could not be determined at the concentrations of magainin II tested on bladder cancer cell lines \(^{(186)}\). Suttmann et al., had reported that fibroblast cell lines 3T6 and ZF07 were significantly less or not at all susceptible to cecropin A and B \(^{(141)}\). No toxicity was reported by Chen et al., with AMP-CM4 against normal cells HEK-293 \(^{(243)}\).

Based on its spectrum of activity, magainin II is highly potent against cancer cells, but not against normal mammalian cells \(^{(244)}\). The lytic action of magainin II is due to the presence of net positive charges of magainin that interacts with the negative charges located in the outer leaflet of the cancer cell membrane \(^{(245)}\). O-glycosylated mucin, a type of glycoprotein, exists in the cancer cell membranes that contribute to the negative charges on the cancer cell surfaces \(^{(246)}\). However, normal mammalian cell membranes are mainly composed of neutral zwitterionic phospholipids and sterols \(^{(102)}\). The net positive charge and hydrophobicity, which have the ability to adopt an amphipathic conformation, are critical structural parameters for antitumor activity \(^{(247,248)}\). Therefore cationic AMP of magainin II is resistant to normal cell lines.
5.3 Apoptosis

Inefficient or defective apoptosis is considered as one of the hallmarks of tumorigenicity (249). Many genes play important roles as regulators of homeostasis between cell death and proliferation (250,251). The studies by Hickman and Kerr demonstrated that the cells undergoing apoptosis exhibit several morphological and cellular changes (252,253).

Apoptosis is characterized by several biochemical criteria, including different kinetics of phosphatidylserine (PS) exposure on the leaflet of the plasma membrane, changes in mitochondrial membrane permeability, caspase activation, internucleosomal DNA cleavage, release of intermembrane space mitochondrial proteins (254) as well as by a series of morphological changes such as formation of apoptotic bodies, nuclear and cytoplasmic condensation, chromatin fragmentation, shrinkage of cells and bleb formation (255).

In the present study on qualitative analysis of DNA fragmentation, a significant increase of DNA fragmentation has been detected in IC\textsubscript{50} of magainin II treated mutant p53 and wild p53 colon cancer cell lines, which corroborates the apoptotic action of magainin II on colon cancer cells. This result suggested that magainin II caused DNA fragmentation characteristic of apoptotic process with the generation of multiple DNA fragments.

A biochemical hallmark of apoptosis was the cleavage of chromatin into small fragments including oligonucleosomes, which were described as DNA ladders in the electrophoresed gel (256). A hallmark of apoptosis is the presence of DNA ladder patterns that originate from DNA cleavage by an endonuclease, which cleaves between the nucleosomes resulting in fragments of multimers of 180 base pairs (257).

It is well known that the activation of endonucleases results in the fragmentation of DNA and can be seen by electrophoretic examination as a characteristic ladder pattern (258). The study by Risso et al., showed that BMAP-28 induced DNA fragmentation in histiocytic
lymphoma U937 cell line (259). Cruz-Chamoro et al., reported that magainin I, nisin induced DNA fragmentation in human promyelocytic leukemia HL-60 cell line (187).

The nuclear morphological changes were investigated either by DAPI or Hoechst staining. After 24 h of treatment at IC\textsubscript{50} concentration of magainin II on mutant p53 and wild p53 colon cancer cell lines, a two-fold increase in apoptotic cells was visible and these cells exhibited apoptotic features such as morphological changes, cell shrinkage, chromatin condensation and fragmentation into discrete bodies. Ceron et al., observed that apoptotic morphological changes were exhibited in HL-60 cells treated with cecropin (138). Huang et al., demonstrated that pardaxin induced apoptosis of HT-1080 cell line examined by nuclear staining (260).

On the basis of overall cell morphology and cell membrane integrity, necrotic and apoptotic cells can be distinguished by AO/EtBr fluorescence staining using fluorescence microscopy. In the present study AO/EtBr staining revealed that IC\textsubscript{50} concentration of magainin II, induced apoptosis on mutant p53 and wild p53 colon cancer cell lines. The apoptotic cells containing the condensed form of nuclei and apoptotic bodies were stained orange whereas the necrotic cells were stained red and the untreated colon cancer cell lines were stained uniform green. Therefore, these results corroborate the involvement of this magainin II in an apoptotic induction rather than in a necrotic action.

Chen et al., demonstrated the variation of morphology in U937 cells after treatment with epinecidin-1 for 24 h stained with AO/EtBr (261). Ceron et al., observed cecropin induced apoptosis in HL-60 cell line stained with AO/EtBr (138).

The mode of cell death induced by magainin II on mutant p53 and wild p53 colon cancer cells were further confirmed quantitatively using Annexin-V/PI dual staining by FACS. Annexin-V/PI staining is able to distinguish early apoptotic cells from late apoptotic
and necrotic cells. At the early stage of apoptosis phosphatidylserine (PS) is translocated from the inner to the outer layer of the plasma membrane which are stained by Annexin-V (262).

In our study magainin II, on mutant p53 and wild p53 colon cancer cells might directly disrupt cell membranes to lysis. Annexin V binds with a high affinity to negatively charged phosphatidylserine, in addition, the externalization of phosphatidylserine from the inner to the outer leaflet in the plasma membrane of tumor cells is an unequivocal indicator of apoptosis induction, which reveals important changes in plasma membrane after incubation in the presence of this magainin II (187). In this particular state, it is possible that cell plasma membranes are more susceptible to magainin II. This results in PS exposure, the release of cytoplasmic contents which leads to cell death. In view of these observations, we could confirm that magainin II mainly induces early apoptosis, and late apoptosis in a low proportion in mutant p53 and wild p53 colon cancer cells.

Zhang et al., demonstrated that percentage of early apoptosis is significantly high in BMAP-28 on thyroid cancer cell lines (263). Gu et al., reported that the number of apoptotic cells increased significantly at an early stage of cell apoptosis in GLI13-8 treated human hepatocellular carcinoma HepG2, human gastric carcinoma SGC7901, and human melanoma A375 cancer cell lines (129). Jin et al., observed early apoptosis in cecropin treated BEL-7402 hepatocellular carcinoma cells (264).

There is a balance between cellcycle arrest and cell death through apoptosis in response to genomic damage and cellular stress in proliferating cells. Defects in this balance lead to the development of cancer and other pathological conditions (265). Cells growth is controlled by cell cycle checkpoints: G1, S, G2/M phase. Cell cycle check point-related mechanisms can be interrupted in cancer by accumulating mutations (266).
In the present study our results indicates an increased G1 phase of cell cycle at IC\textsubscript{50} concentration of magainin II for 24h on mutant and wild p53 colon cancer cell lines, whereas 5-FU induced S phase of cell cycle arrest in mutantp53 and wildp53 colon cancer cell lines. The activity of magainin II on cell cycle arrest might be due to its efficacy in inhibiting CdKs, thereby resulting in reduction in number in colon cancer cell lines.

The study by Lu et al., showed that PFR peptide induced cell cycle arrest in MEL cells as evidenced by significant increase of cell population in G0/G1 phase after 24 h treatment \(^{(123)}\). Chen et al., observed that G1 population increases after 24 h treatment of KL15 on human adenocarcinoma colon cells SW480 and Caco-2 cell lines \(^{(267)}\). Zhao et al., demonstrated that HPRP-A2 was blocked in G1 phase of cell cycle on BGC-823 cells \(^{(126)}\).

### 5.4 ROS generation

In living organisms, an adequate increase in ROS can encourage proliferation and differentiation, whereas excess ROS generation causes oxidative damage to cells and induce cell apoptosis \(^{(268–270)}\). ROS play a key role by regulating apoptotic signalling and activating certain enzymes involved in the cell death pathway \(^{(271)}\). The ROS generation and the sequence of events such as oxidative stress and mitochondrial disruptions have been suggested as components of a final common pathway during execution of apoptotic program \(^{(272)}\).

In the present study, the qualitative and quantitative analysis of ROS generation suggest that magainin II induced significantly increased level of ROS generation in wild p53 colon cancer cell line, whereas in mutant p53 colon cancer cell lines marginal increased level of ROS generation. This indicated that magainin II is involved in apoptotic induction, because ROS generation has been demonstrated to be an early signal that mediates apoptosis\(^{(273)}\). It is well established that numerous apoptotic stimuli, including ROS
generation, initiate apoptosis by disturbing mitochondrial function sufficiently to trigger leakage of cytochrome-C from mitochondria \(^{(274)}\). Mounting evidence proved that the increasing ratio of Bax and Bcl-2 could decrease MMP, induce the release of cytochrome-C, and eventually result in ROS accumulation \(^{(275)}\).

The study by Wang et al., demonstrated that temporin1CEa, triggered a rapid cell death in MCF-7 and MDA-MB-231 cells through ROS over production \(^{(276)}\). Zhao et al., showed that HPRP-A2 induced apoptosis in BGC-823 cells involves a marked increase in generation of reactive oxygen species \(^{(126)}\). Paredes-Gamero et al., reported that β-hairpin AMPs Gomesin and protegrin induced cell death in the human erythroleukemia K562 cell through apoptosis dependent on intracellular Ca\(^{2+}\) mechanisms and the participation of free radicals \(^{(277)}\).

### 5.5 Activation of Caspase -3

Caspase-3 is a key member of the caspase family, a group of cysteine proteases that mediate apoptotic execution \(^{(278)}\). Caspase-3 plays a very important role in the cancer cell apoptosis process \(^{(279)}\). It can be activated by apoptotic signals from both death receptor and intracellular/mitochondrial pathways. Caspase-3 functions as a major effector caspase by cleavage of numerous cell death substrates, to cellular dysfunction and destruction \(^{(278)}\). Caspase-3 deficiency and down regulation have been associated with carcinogenesis, suggesting caspase-3 could be a biomarker in cancer prevention and treatment \(^{(280)}\).

In the present study, magainin II treatment significantly increased the activity of caspase-3 in both mutant p53 and wild p53 colon cancer cell lines. The result suggest that magainin II induced caspase-dependent apoptosis in both mutant p53 and wild p53 colon cancer cell lines.
The result was supported by Zhang et al., who reported that the protein and mRNA expression of caspase-3 and caspase-9 increased significantly in the BMAP-28-treated thyroid cancer TT cells, which indicated that BMAP-28 could induce apoptosis in the TT cells via the activation of caspase-9 and caspase-3 (263). Likewise Huang et al., showed that pardaxin triggers caspase-dependent and ROS-mediated apoptosis in HT-1080 cells (260).

5.6 Mitochondrial membrane potential (MMP)

Mitochondria provides energy in the form of ATP which is required for cells to die by the apoptotic pathway, and release proapoptotic proteins normally attached in the intermembrane space into the cytosol, in which they trigger downstream apoptotic signalling pathways (281–284). Most apoptotic pathways converge on the mitochondria, inducing the disruption of the mitochondrial transmembrane potential \(\Delta \phi_m\). The loss of mitochondrial membrane potential (\(\Delta \phi_m\)) is an early and already irreversible stage of apoptosis (285). Moreover, mitochondrial dysfunction during apoptosis is very often associated with the activation of caspases (286). The reduction of membrane potential leads to the release of mitochondrial cytochrome-C, which is the key factor that results in the formation of apoptosomes (287,288).

In the present study, we have observed an increased depolarization of the mitochondrial membrane potential in IC\(_{50}\) magainin II treated mutant p53 and wild p53 colon cancer cell lines. Magainin II induces a disturbance in the mitochondrial transmembrane potential thereby transducing the apoptotic signal through the mitochondrial pathway. These result suggest that apoptosis induced by magainin II is dependent on the mitochondrial membrane integrity, which has been reported to be disrupted due to opening of the permeability transition pores. Disruption of the mitochondrial membrane potential is thought to be a common event in the induction of apoptosis (289). Production of MMP across the
mitochondrial inner membrane is the result of utilization of oxidizable substrates, therefore excessive generation of ROS in cells may lead to the loss in MMP\(^{(290)}\).

Wang et al., showed that that temporin-1CEa induced rapid cell death through mitochondria-involved mechanisms, including rapid intracellular Ca\(^{2+}\) leakage, collapse of mitochondrial membrane potential and over-generation of reactive oxygen species (ROS) in Bcap-37 human breast cancer cells. Rissø et al., showed that BMAP-28 induced the mitochondrial permeability transition pore and its cytotoxic potential depends on its effects on mitochondrial permeability\(^{(131)}\). Cerón et al., demonstrated that cecropin A on human promyelocytic leukemia cells triggered rapid cell death through the loss of mitochondrial transmembrane potential and generation of reactive oxygen species\(^{(138)}\).

Libério et al., observed apoptosis in pentadactylin treated murine melanoma cell line B16F10 associated with alteration of mitochondrial membrane potential\(^{(291)}\). Hilchie et al., showed that NRC-03 and NRC-07 on breast cancer cell lines MDA-MB-231, MDA-MB-468, T47-D, SKBR3, MCF7, caused mitochondrial transmembrane potential and induced ROS production\(^{(292)}\). Zhao et al., demonstrated that HPRP-A2-induced cell death is associated with the generation of reactive oxygen species, the depolarization of MMP and the activation of the caspase activities\(^{(126)}\).

5.7 Gene Expression

Apoptotic cells induce two major pathways: the extrinsic pathway and intrinsic pathway. In the extrinsic pathway, signal molecules trigger via the death receptor on the cell membrane and activate by extra cellular ligands\(^{(293)}\). The intrinsic pathway is activated by cellular stress, particularly mitochondrial stress caused by DNA damage and heat shock\(^{(294)}\). The death signals is determined by the ratio between pro and anti-apoptotic proteins which then leads to apoptosis.
Apoptosis is induced by p53 via transcriptional up-regulation of the pro-apoptotic Bax and by inhibition of the anti-apoptotic Bcl-2 gene (295). The absence of pro-apoptotic Bax mRNA expression in colorectal cancer cells can induce resistance to apoptosis triggered by different chemotherapeutic agents (296–298). FasL is implicated in the induction of apoptosis (299). Defects in the Fas/FasL apoptotic signalling pathway provide a survival advantage to cancer cells and may be implicated in tumorigenesis (300,301).

In the present study, using semi quantitative RT-PCR, magainin II showed that marginal increased mRNA expression of p53, upregulation of Bax, repression of Bcl-2 mRNA expression and no changes of FasL mRNA expression in mutant p53 colon cancer cell lines. But whereas in wild p53 colon cancer cell line, our results revealed that magainin II showed significant increased mRNA expression of FasL, p53, Bax and decreased mRNA expression of Bcl-2. The results suggest that magainin II activates p53 mRNA expression and may trigger apoptosis via induction of Bax, inhibition of Bcl-2 and the activation of caspase-3 in mutant p53 colon cancer cell line. In wild p53 cell line, magainin II induced cell death involving p53 and Bax mRNA expression mediated by FasL.

Jin et al., demonstrated that cecropin induced extrinsic pathway in human hepatocellular carcinoma cell line BEL-7402 cells by increasing the level of Fas, Fas-L, caspase-8 and caspase-3 gene expression levels (264). Lo et al., showed that hepcidin on human cervical cancer HeLa, SCC15, and NT2D1 cell lines had significantly raised the mRNA expressions of p53, Bax and the ratio of Bax/Bcl-2 (302). Tong-ngam et al., showed that BmKn-2 peptide, possessed apoptogenic effect on HSC-4 oral cancer cell line by increasing the expression levels of pro-apoptotic genes such as caspase-3, -7, and -9 and depressed the mRNA expression level of anti-apoptotic Bcl-2 (303). Tsai et al., reported that m2163 and m2386 induced both the extrinsic and intrinsic apoptosis pathway by increased the expression of Fas and TRAILR1 death receptors on the cell surface of treated SW480
colon cancer cells and also increased the expression of mitochondria-related apoptosis proteins such as Smac\(^{(304)}\).

5.8 Protein expression

The tumor-suppressor protein p53 accumulates in cells in response to DNA damage, oncogene activation, and other stress factors. Depending on the cellular context, activation of p53 could lead to cell-cycle arrest, apoptosis, cellular senescence, differentiation, and autophagy\(^{(305)}\). Activation of p53 can trigger both the mitochondrial (intrinsic) and the death-receptor-induced (extrinsic) apoptotic pathways\(^{(306)}\). p53 protein was detected by conventional immunohistochemistry in 42% of 52% colorectal adenocarcinomas\(^{(307)}\).

In the present study, magainin II treated mutant p53 colon cancer cell lines showed a marginal increase in p53, activates intrinsic pathway and wild p53 colon cancer cell line showed significant increase in p53 protein activates both intrinsic and extrinsic pathway. These results are consistent with previous result, which suggested up regulated expression of p53 after FK-16 treated colon cancer cells. FK-16 activated nuclear p53 to upregulate Bax and downregulate Bcl-2\(^{(308)}\). Ren et al., demonstrated that LL-37 increased the level of p53 protein expression, induced caspase-independent apoptosis in human colon cancer cells via the activation of G1-coupled GPCR-p53-Bcl-2/Bax/Bak-AIF/EndoG cascade in human colon\(^{(309)}\).

Our results showed that the treatment of mutant and wild p53 colon cancer cell lines with magainin II released cytochrome-C from the mitochondria into the cytosol, suggesting an activation of the intrinsic apoptotic pathway. Mitochondria have been shown to play a central role in the apoptotic process because both the intrinsic and extrinsic pathways can converge at the mitochondrial level and trigger mitochondrial membrane permeabilization\(^{(310,311)}\). After apoptotic-stimulated mitochondrial membrane permeabilization, cytochrome-C and other mitochondrial pro-apoptotic factors are released
into the cytosol. The cytochrome-C thus released subsequently triggers the activation of caspases, the induction of the apoptotic process and subsequent cell death. Cytochrome-C binds to Apaf-1 and procaspase-9 to form the apoptosome, activating caspase-9, the primary caspase involved in the mitochondrial apoptotic pathway \(^{(312)}\).

Risso et al., demonstrated that BMAP-28 treatment of U937 and K562 human leukemia cell lines caused a rapid reduction in mitochondrial membrane potential in situ that was related to a BMAP-28-induced opening of the mitochondrial permeability transition pore, which resulted in cytochrome-C release and the initiation of cell death by apoptosis \(^{(259)}\). Cruz-Chamoro et al., showed that magainin 1 induced apoptosis in HL-60 human promyelocytic leukemia cells via a mechanism that involves cytochrome-C release from mitochondria and an increase in proteosome activity \(^{(187)}\).

p53 induces the expression of pro-apoptotic Bcl-2 (B-cell lymphoma-2) family proteins, mainly Bax, but downregulates the anti apoptotic protein Bcl-2, leading to permeabilization of outer mitochondrial membrane. Then cytochrome-C releases from the mitochondria binds to Apaf-1 and induces the activation of the initiator caspase-9, eventually resulting in the activation of executioner caspase-3, -6 and -7 \(^{(313)}\).

Mitochondrial disorganisation is usually mediated by the participation of the Bcl-2 protein group. Bcl-2 and Bax have been identified as major regulators in controlling the release of mitochondrial cytochrome-C \(^{(314,315)}\). Bcl-2 binds to the mitochondrial outer membrane, thus blocking cytochrome-C efflux. In contrast, Bax translocates from the cytosol to the mitochondria where it enhances the release of cytochrome-c and encourages apoptosis. Many anticancer agents or apoptotic stimuli can trigger the release of cytochrome-C through either the down-regulation of Bcl-2 and/or up-regulation of Bax \(^{(312)}\).
In the present study, the results showed that magainin II up-regulated the expression of Bax and downregulated the expression of Bcl-2 in mutant and wild p53 colon cancer cell lines.

Xia et al., showed that CecropinXJ induced caspase-dependent apoptosis in the human hepatocellular carcinoma Huh-7 cell line by upregulated Bax, Bad expression, downregulated Bcl-2 expression and cleaved caspase and PARP in a time dependent manner (316). Jin et al., reported that cecropin induced apoptosis by triggering extrinsic apoptotic pathway in human hepatoma BEL-7402 cells, which might be associated with up-regulation of Fas, Fas-L, caspase-8, and caspase-3 (264).

5.9 Telomerase activity

Telomeres alterations have been described in many diseases including aging-related disease (317–319). Telomerase, which is responsible for telomere lengthening, plays a pivotal role in tumorigenesis (320–322). Telomerase activity is undetectable in most normal somatic cells except embryonic cells and germline cells (323). In colon cancer, telomerase is activated very early in the process of the disease suggesting that its activation might be also a determining factor that contributes to the process of tumorgenesis (63).

In the present study, we found that the activity of telomerase by real time PCR was reduced after magainin II treatment in mutant p53 and wild p53 colon cancer cells. Our results indicated that magainin II showed a significant repression on telomerase activity in wild p53 colon cancer cell line. Telomerase activity may also be an indicator of responsiveness to cytotoxic agents. The results suggests that telomerase actvity is reduced by the activation p53, increased ROS and decreased Bcl-2 expression. The results of the present study are in concordance with the study by Jana et al., who demonstrated that LL-37
is a binder of the telomeric G-quadruplex, appears to stabilize G-quadruplexes and thereby reduces telomerase activity in cancer cells \(^{(324)}\).

Telomerase inhibitors can promote apoptosis in many hematologic malignancies such as myeloma, and inhibition of telomerase results in telomere shortening, repressed proliferation and altered cell cycle that results in apoptosis in cancer cells \(^{(325,326)}\). This indicates that anti-telomerase therapy can not only enhance apoptosis in tumor cells, but may also be one of the most important and effective markers for the selection of new anti-tumor drugs \(^{(327,328)}\). Sulforaphane decreases viability and telomerase activity in hepatocellular carcinoma Hep3B cells through the reactive oxygen species-dependent pathway \(^{(329)}\).

Activated \(p53\) has been associated with regulation of the telomerase activity \(^{(330–332)}\).