



## Research Article

# The Angiotensin-Converting Enzyme Inhibitor ‘Enalapril’ Increases the Anti-Proliferative Activity of 5-Fluorouracil in Colorectal Cancer Cells

 Asma Mostafapour,<sup>1</sup>  Javad Baharara,<sup>2</sup>  Majid Khazaei,<sup>3</sup>  Amir Avan,<sup>4</sup>  Seyed Mahdi Hassanian<sup>5</sup>

<sup>1</sup>Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran

<sup>2</sup>Department of Biology & Research Center for Animal Development Applied Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran

<sup>3</sup>Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

<sup>4</sup>Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

<sup>5</sup>Department of Medical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

### Abstract

**Objectives:** The dysregulation of angiotensin-converting enzyme inhibitor (ACE-I) and its pathway has been reported to be associated with oncogenesis and poor prognosis in colorectal cancer (CRC).

**Methods:** We explored the therapeutic potential of targeting ACE-I through the use of enalapril and investigated its pharmacological interaction with 5-FU in CT26, HT29, and SW480 cells. The anti-proliferative and anti-migratory effects as well as apoptotic activity of this agent on cell cycle have been evaluated by MTT, wound healing assay, and FACS, while the expression of genes was determined through mRNA or protein levels.

**Results:** According to the observations, enalapril has inhibited cell proliferation in a dose-dependent manner and affected the anti-tumor properties of 5-FU via increasing the levels of apoptosis and ROS, as well as through the modulation of antioxidant/oxidant markers. Enalapril has also suppressed cell migration by the perturbation of MMP3/MMP-9 and E-cadherin. The combination of enalapril and 5FU has resulted in decrease in the expression levels of ACE, AT1R, and SMAD-3.

**Conclusion:** Our findings suggested that targeting ACE-I by applying the modulation of angiotensin pathway could increase the activity of 5-FU due to interfering with cell-proliferation, apoptosis, and inflammatory markers, which is indicative of its potential value as a therapeutic option for the treatment of CRC.

**Keywords:** ACE inhibitor, Enalapril, Colorectal Cancer, Combination therapy

**Cite This Article:** Mostafapour A, Baharara J, Khazaei M, Avan A, Hassanian SM. The Angiotensin-Converting Enzyme Inhibitor ‘Enalapril’ Increases the Anti-Proliferative Activity of 5-Fluorouracil in Colorectal Cancer Cells. *EJMO* 2021;5(4):318–326.

Cancer is typically distinguished by detecting its progressive and degenerative effects. It can also be characterized by abnormal control mechanisms that affect cell survival, proliferation, and differentiation.<sup>[1]</sup> Colorectal cancer is one of the important causes of mortality and leads to 500,000 deaths annually worldwide.<sup>[2]</sup> However, although patients are treated with the combination of different che-

motherapeutic agents, most of them eventually become resistant to the treatments.<sup>[3, 4]</sup> An angiotensin-converting enzyme inhibitor (ACE-I), Enalapril, is exerted in clinical practice throughout the treatment of several conditions, including hypertension and heart failure.<sup>[5]</sup> This substance can reduce cell proliferation and cause cell death by inducing cellular signaling pathways that involve TGF-beta as the main regula-

**Address for correspondence:** Javad Baharara; Majid Khazaei, MD. Department of Biology & Research Center for Animal Development Applied Biology, Mashhad, Iran; Ferdowsi University Pardis, Mashhad, Iran

**E-mail:** baharara@mshdiau.ac.ir; khazaeiM@mums.ac.ir

**Submitted Date:** August 08, 2021 **Accepted Date:** September 15, 2021 **Available Online Date:** December 01, 2021

©Copyright 2021 by Eurasian Journal of Medicine and Oncology - Available online at [www.ejmo.org](http://www.ejmo.org)

**OPEN ACCESS** This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



tor and suppressor factor of tumor in epithelial cells.<sup>[4]</sup> Moreover, enalapril plays a role in cell differentiation, extracellular matrix formation, and immune response.<sup>[3, 6]</sup> However, the recent evidences have exposed the antitumor effect of ACE and ARB inhibitors in different malignancies.<sup>[7, 8]</sup> According to available reports, ARB inhibitors can cause a decrease in the VEGF expression and an increase in apoptosis.<sup>[9-11]</sup> Besides, it has been indicated by tumor sorts and phases that TGF- $\beta$  signaling has an important and complicated functionality throughout the CRC Progression.<sup>[12-14]</sup> The lack of Smad4 can cause an increase in VEGF expression within the CRC cells.<sup>[15]</sup> However, a high expression rate of VEGF can be related to an extreme vascular solidity, expansion of metastasis, and resistance to medicines, leading to a worse prognosis in CRC.<sup>[16]</sup> Several articles have suggested that ACE-I can decrease tumor extension and metastasis.<sup>[17]</sup> In addition, it has been reported that TGF- $\beta$  receptor is regulated by these inhibitors.<sup>[8]</sup> Angiotensin-2 is capable of enhancing the extension of numerous tissues, including liver, kidney, lung, and heart,<sup>[7]</sup> and advancing its functionality in regards to other cell growth factors.<sup>[9]</sup> In this study, we evaluated the anti-tumor effects of enalapril and 5-FU combination for the purpose of investigating the cellular and molecular pathways that are involved in colorectal cancer cells.

## Methods

### Chemicals and Drugs

Enalapril was provided from Huahaipharm in China, and dissolved in methanol. 5-Fluorouracil (5-FU) was obtained from ebewe PHARMA Ges (Unterach, Austria) and dissolved in distilled water, and we purchased RPMI-1640 medium, Fetal Bovine Serum (FBS), penicillin/streptomycin (pen-strap; 50  $\mu$ g/mL) from in vitro gene (Gaithersburg, MD).

### Cell Culture

The required colorectal cancer cells CT26, HT29, and SW480 were obtained from Pasteur Institute and were incubated in the RPMI-medium and DMEM-medium that contained 10 % FBS and 1% pen-strap, which were then maintained at 37 °C and 5 % CO<sub>2</sub>.

### Growth Inhibition Studies

The cytotoxic activity of drugs was evaluated by the means of MTT assay. Briefly, we plated the cells ( $2 \times 10^4$ ) in 96-wells and treated them with different concentrations of enalapril, 5-FU, and enalapril+5-FU combination (1mM, 500 $\mu$ M, 300 $\mu$ M, 100 $\mu$ M, 10 $\mu$ M, 500nM, 100nM, 50nM, 10nM) for 72 h. Then, the plate was read at 570 nm through the use of an epoch spectrophotometer<sup>[11]</sup> and the value of IC<sub>50</sub> was examined by Prism software.

### Multicellular Spheroids

The cytotoxic activity of utilized drug was assessed in a 3-D cell culture model of CT26 cells. For this purpose, we generated the spheroids by plating 10<sup>5</sup> cells/mL in agarose coated 96-well plates and performed a thorough examination under microscope. The volume of spheroid (V) was determined by the following formula:  $D = (D_{\max} + D_{\min})/2$ , as follows (Eq. 1):

$$V = \frac{4}{3} \pi \left(\frac{D}{2}\right)^3 \quad (1)$$

### Migration Assays

The ability of enalapril, 5-FU, and enalapril+5-FU in suppressing the migratory was investigated by the use of starch assay. A monolayer of CT-26 cells was scratched with a pipette. 24h later, the required images were taken at different time intervals as follows; 12, 24, 36, 48, and 72h.

### Quantitative RT-PCR

Initially, RNAs were extracted from the cells that were treated with enalapril at IC<sub>50</sub> through the utilization of RNXPLoS (CinaColon, Tehran, Iran). Once the cDNA was synthesized (CinaColon, Tehran, Iran), we amplified it with specific primers for ACE, AT1-R, TGF- $\beta$ 1, VEGF A, Smad3, and MMP-3 and MMP-9 genes (Macrogen Co, Seoul, Korea) by exerting the ABI StepOne instrument (Applied Biosystems, Foster City, CA). The 2- $\Delta\Delta$ CT method was extensively used as a relative quantification strategy for quantitative real-time polymerase chain reaction (qPCR) data analysis. The house-keeping gene GABDH were used.

### Apoptosis Analyses

Annexin-V–fluorescein isothiocyanate (FITC) and propidium iodide (PI) assay kits (Cayman chemical Co, Michigan) were used to assess apoptotic cell death. Briefly, the cells of CRC were distributed at a density of  $2 \times 10^6$  cells per well in 6-well plates, and treated with enalapril, 5FU and combination of enalapril and 5FU for 48 hr. The cells extracts were suspended in 200  $\mu$ L 1X Annexin V binding buffer. After centrifuging cells (for 5 minutes at 400 g), the cell pellets were re-suspended in Annexin V-FITC/PI staining solution and were incubated at room temperature for 15 minutes. Subsequently, the rate of viable, secondary necrotic cells, early and late apoptotic cells was quantified using FACS-Calibur flow cytometer (BD Biosciences-US) and FlowJo software.

### DAPI Staining

DAPI (4', 6-diamidino-2-phenylindole dihydrochloride) staining was used to assess the morphology of nuclei

$1.5 \times 10^5$  cells. To perform this procedure, the cells were plated in 6-well plate and treated with enalapril, 5-FU, and enalapril+5-FU combination, which were then incubated for 48h. Thereafter, we washed them with PBS and appended DAPI to have the cells incubated for 10 min. After another step of washing, the cells were suspended in 1000  $\mu$ L of ethanol to observe the nuclei morphology under fluorescence microscopy (Olympus, Japan).

### Caspase-3 and -9 Activity

We exerted a Bio Assay Kit (Abcam-UK) to assess the Caspase -3/9 activity. In this process,  $3 \times 10^6$  cells were treated with the  $IC_{50}$  value of drug for 48 h, which were then harvested and mixed with 50  $\mu$ L of cell lysis buffer. As the next step, 50  $\mu$ L of reaction buffer plus 10 mM DTT was appended. After being mixed with 5  $\mu$ L of the substrates (4 mM), it was incubated for 1 h at 37 °C and recorded the absorbance at 405 nm.

### Reactive Oxygen Species (ROS) Evaluation by DCFH-DA Method

The evaluation of intracellular ROS production was performed by measuring the induced change in the absorbance of redox-sensitive dye 2', DCFH-DA, and it was converted to a green highly fluorescent product, DCF. Briefly, the CRC cells were treated with enalapril, 5-FU, and enalapril+5-FU combination for 6 h within a complete medium. Then, the cells were incubated in a fresh culture medium that contained DCFH-DA for 30 min. After being washed for three times with PBS, we measured the fluorescence values by the use of a fluorimeter and the images of the cells were taken by an inverted fluorescence microscope.

### Cytokine Detection Assays

The Quantification of IL-6 and TNF- $\alpha$  in colon cancer cells was performed through the use of cytokine ELISA kits (bioscience), while keeping the process in accordance with the manufacturers' protocol.

### Measurement of MDA

We measured the MDA level of the cells that were treated with enalapril, 5-FU, and enalapril+5-FU, which was previously described. Shortly, 1 mL of the 10% homogenates sample was added to 2 mL of TBA+TCA+HCl solution for 45 min and then was centrifuged for 10 min. In the following, the mixture was measured at 535 nm and the level of MDA was calculated by applying the given equation (Eq. 2):

$$C_{(M)} = \frac{A}{1.65} \times 10^5 \quad (2)$$

### Assessment of Total Thiol Groups

The total thiol was evaluated by the application of DTNB. In detail, 1 mL of Tris-EDTA at the pH 8.6 was mixed with the designated samples and read at 412 nm (A1). Then, we have added 20  $\mu$ L of DTNB to the solution, while continuing the process by incubation for 15 min and recording again as A2. The DTNB absorbance was considered to be blank (B). It should be noted that the level was calculated by  $(A2 - A1 - B) \times (1.07/0.05 \times 13.6)$ .

### SOD Activity

Superoxide dismutase was evaluated by the utilization of Ransod kit (Randox Laboratory, London, UK), and the Enzyme response velocity was evaluated through the conversion of  $H_2O_2$  to  $H_2O$  and  $O_2$  as described.

### Statistical Analysis

The given data was presented as mean  $\pm$  SEM. SPSS-20, while Prism was applied to assess the statistical significance and it was set as  $p \leq 0.05$ .

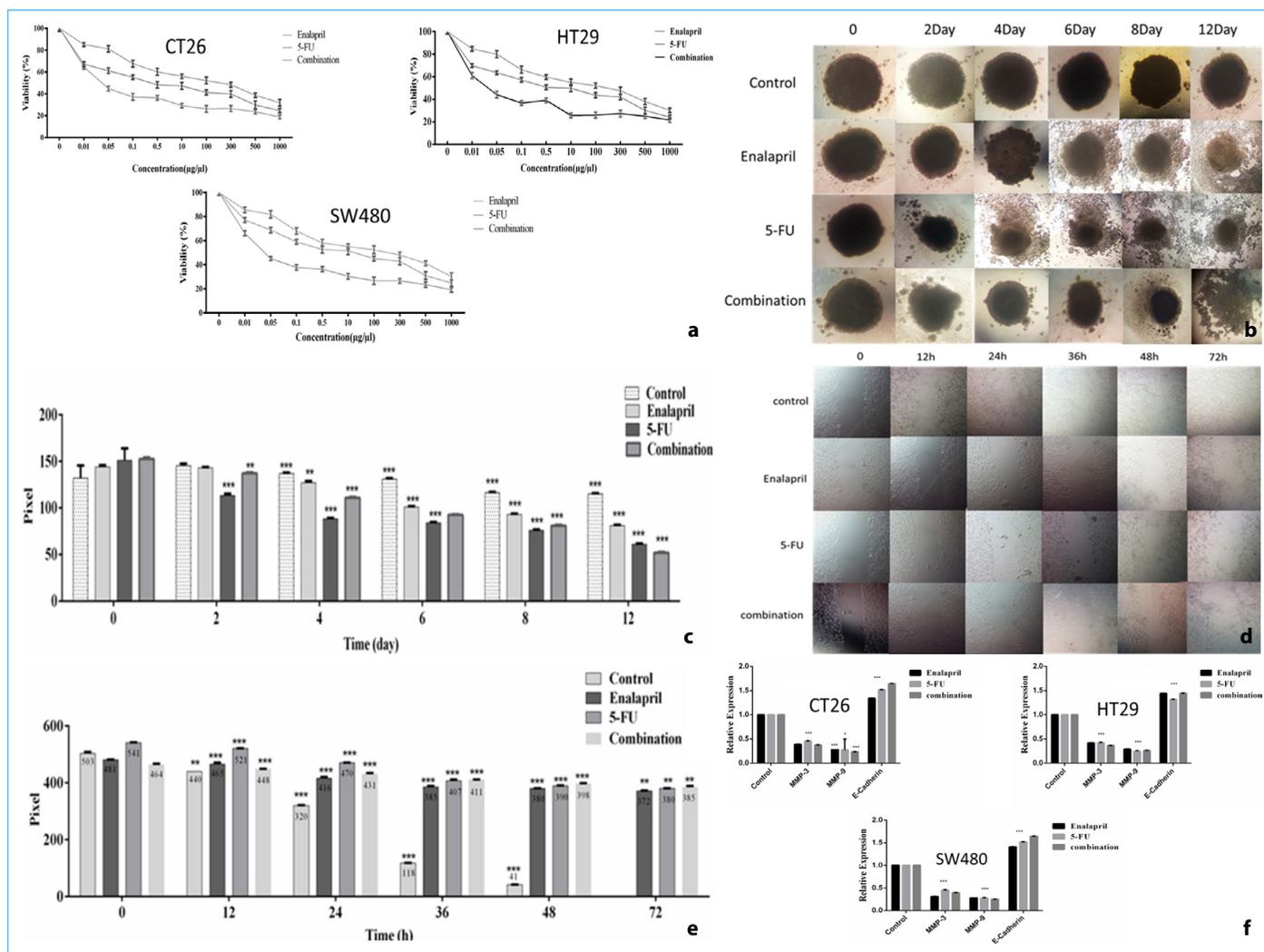
## Results

### Enalapril Reduced Cell Growth and the Migratory Behavior of Cells

According to the assessment of the growth inhibitory effect of enalapril, 5-FU, and enalapril+5-FU combination, it was indicated that an increase in the enalapril concentration could result in a decrease in the cell growth (Fig. 1a). In order to explore whether enalapril was also active in 3D cell models and it was capable of overcoming the major obstacles in conventional methods, we developed spheroids and treated them with enalapril at  $IC_{50}$  (235  $\mu$ g/mL) values. According to the outcomes, enalapril was able to reduce the size of tumor after 8 days, which was exhibited in Figure 1 b-c. Furthermore, we investigated the effects of enalapril on migratory behaviors (Fig. 1d-e). The obtained data revealed a significant reduction in the migration of CRC cells after 20 hours. In the following, the expression level of E-cadherin, MMP-3, and MMP-9 was evaluated (Fig. 1f) and it could be stated that this drug was able to suppress the expression of MMP-3 and MMP-9, while increasing the level of E-cadherin.

### Effects of Enalapril on Increasing Apoptosis

In this part of our work, we determined the apoptotic activity of enalapril in CRC cells through the DAPI staining, which provided some information on nuclear apoptosis and DNA fragmentation. According to our data, although the treated cells exhibited cell death in higher enalapril concentrations, it was more pronounced in the case of



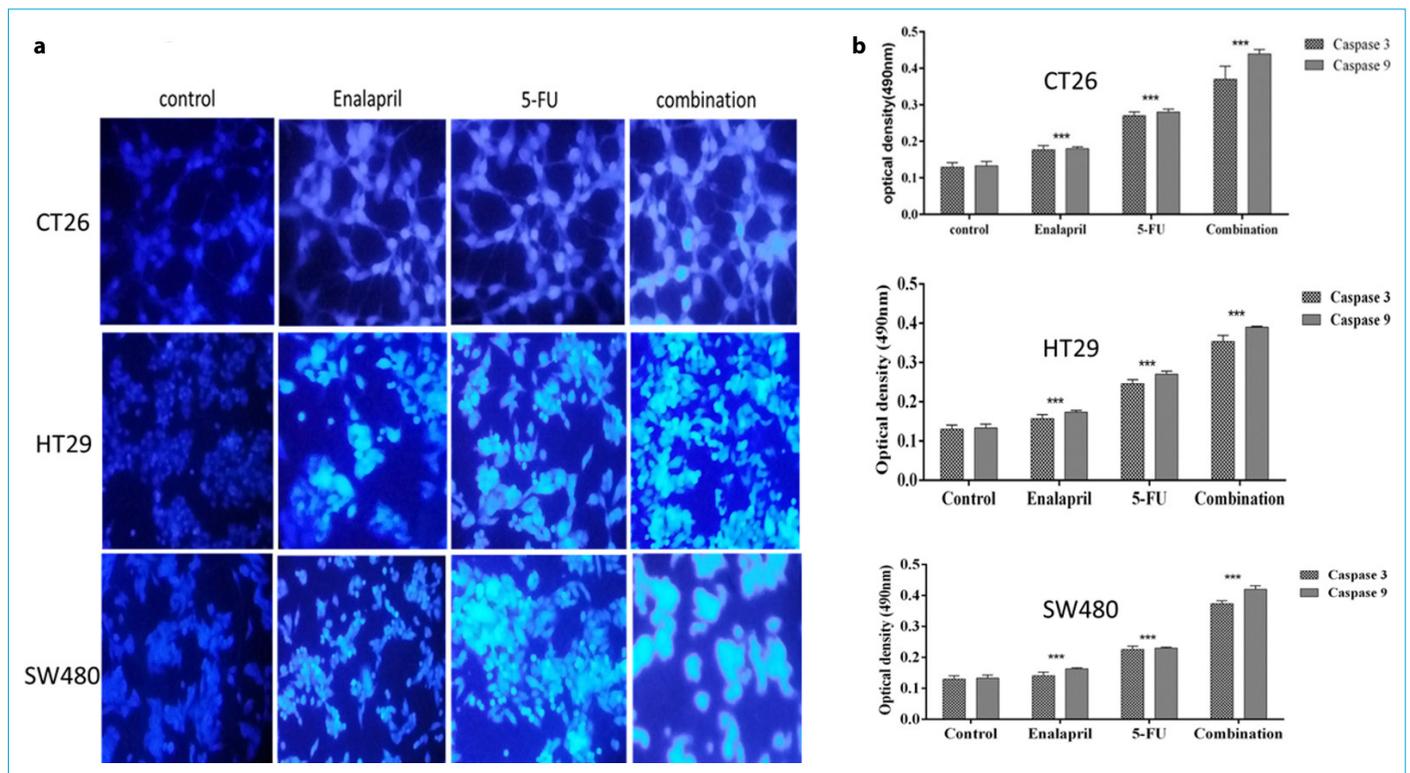
**Figure 1.** Inhibition of cell proliferation and migration in colorectal cancer cells; **(a)** Growth inhibitory effects after 72 h exposure to enalapril and 5-FU in CRC cells, **(b, c)** Effect of enalapril and 5-FU on the CT26 cells spheroids, **(d, e)** Migration of CT26 cells with enalapril and 5-FU, and **(f)** Modulation of E-cadherin and MMP3/9 mRNA levels in CRC cells after 24 h exposure to enalapril and 5-FU as determined by qRT-PCR. Columns or points, mean values obtained from three independent experiments; Error bars, standard error of the mean. \*Significantly different from controls (c).

enalapril plus 5-FU combination (Fig. 2a). Furthermore, the level of caspase activation was calculated by the means of calorimetrically assay (Fig. 2b) and the outcomes suggested that enalapril could exceed the cytotoxic functionality of 5-FU by increasing the caspase3/9 activity in treated cells, as compared with the control group. Also, we found that enalapril, 5-FU, and combination increased in both early (annexin V positive/ PI negative) and late (annexin V and PI positive) apoptosis (Fig. 3).

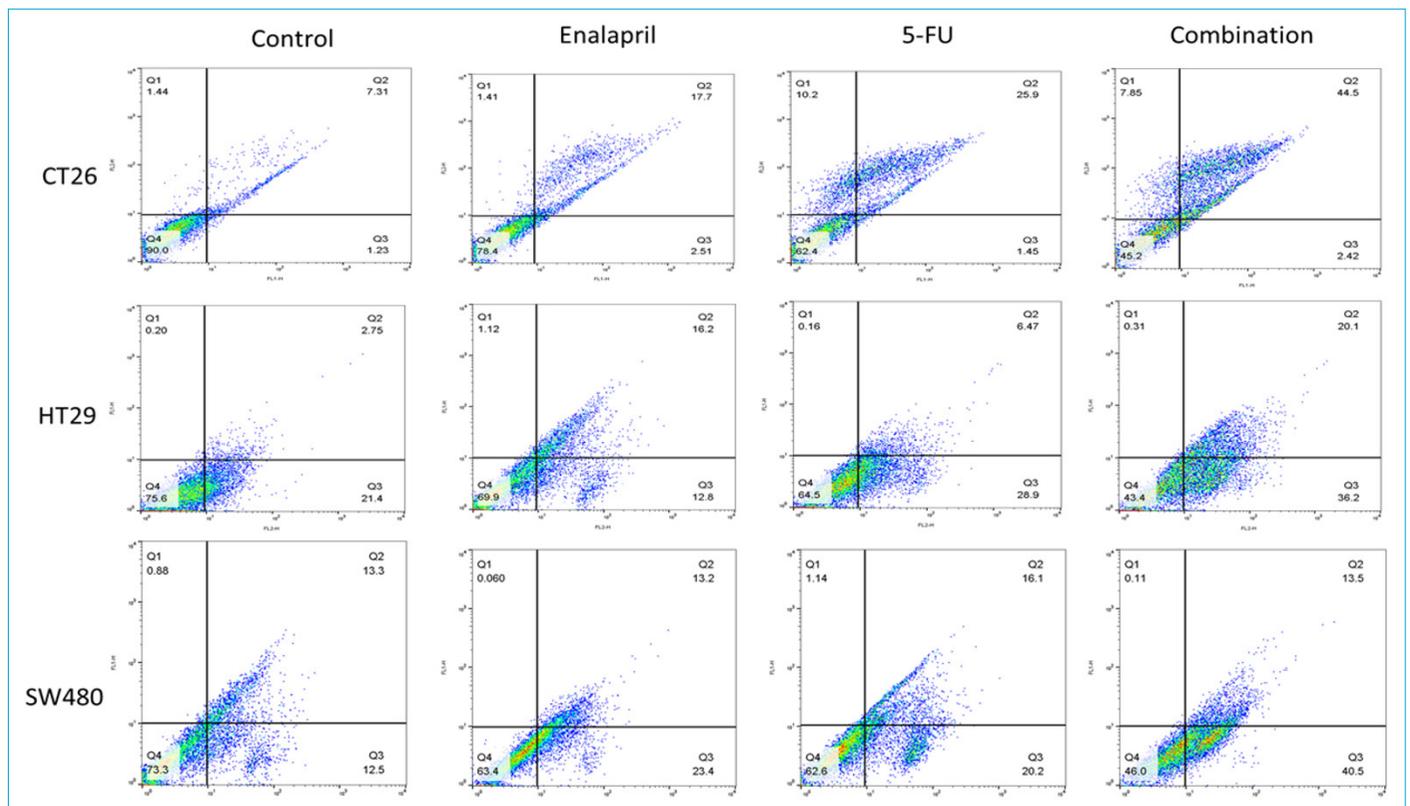
### Enalapril in Combination with 5-FU Increased ROS Generation, Modulate Antioxidant/Oxidant, and Cytokines Factors

Our results indicated that enalapril could increase the generation of ROS in CRC cells, which can be detected

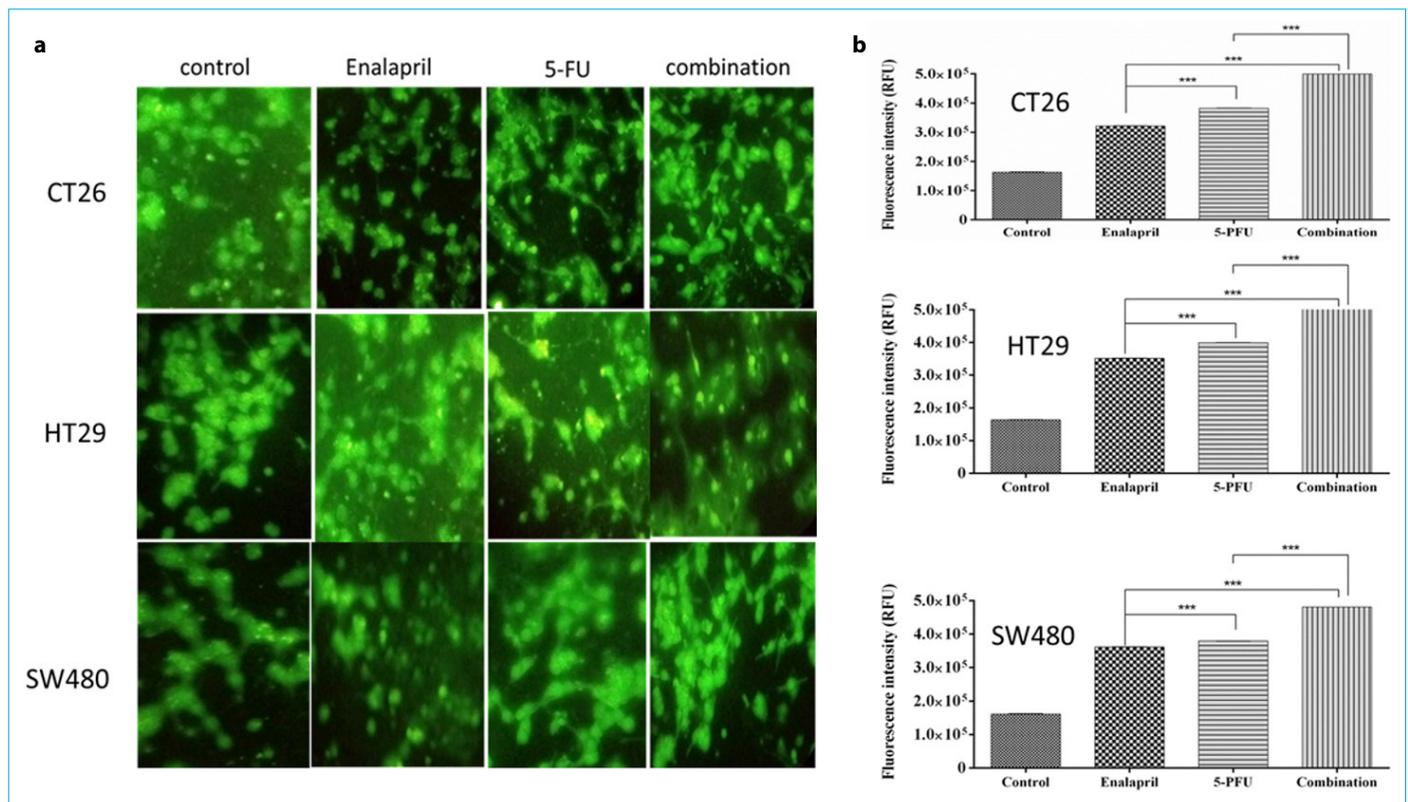
through the measurement of DCFH-DA staining (Fig. 4a-b). These outcomes suggested that enalapril could disrupt the antioxidant/oxidant balance and cause more ROS production, resulting in increased damage and consequently leading to a higher number of CRC cell death. To further assess the role of enalapril in oxidative stress, we had to measure the concentrations of SOD, MDA, and thiol (Fig. 5). The level of MDA was increased, while the level of SOD and total thiol were decreased in the CRC cells that were treated with enalapril. Moreover, we explored the effect of enalapril and its combination with 5-FU on expression levels of TNF- $\alpha$  and IL-6. According to the results, the administration of enalapril+5-FU caused a decrease in the expression level of inflammatory cytokines (Fig. 6b), suggesting that this drug could attenuate



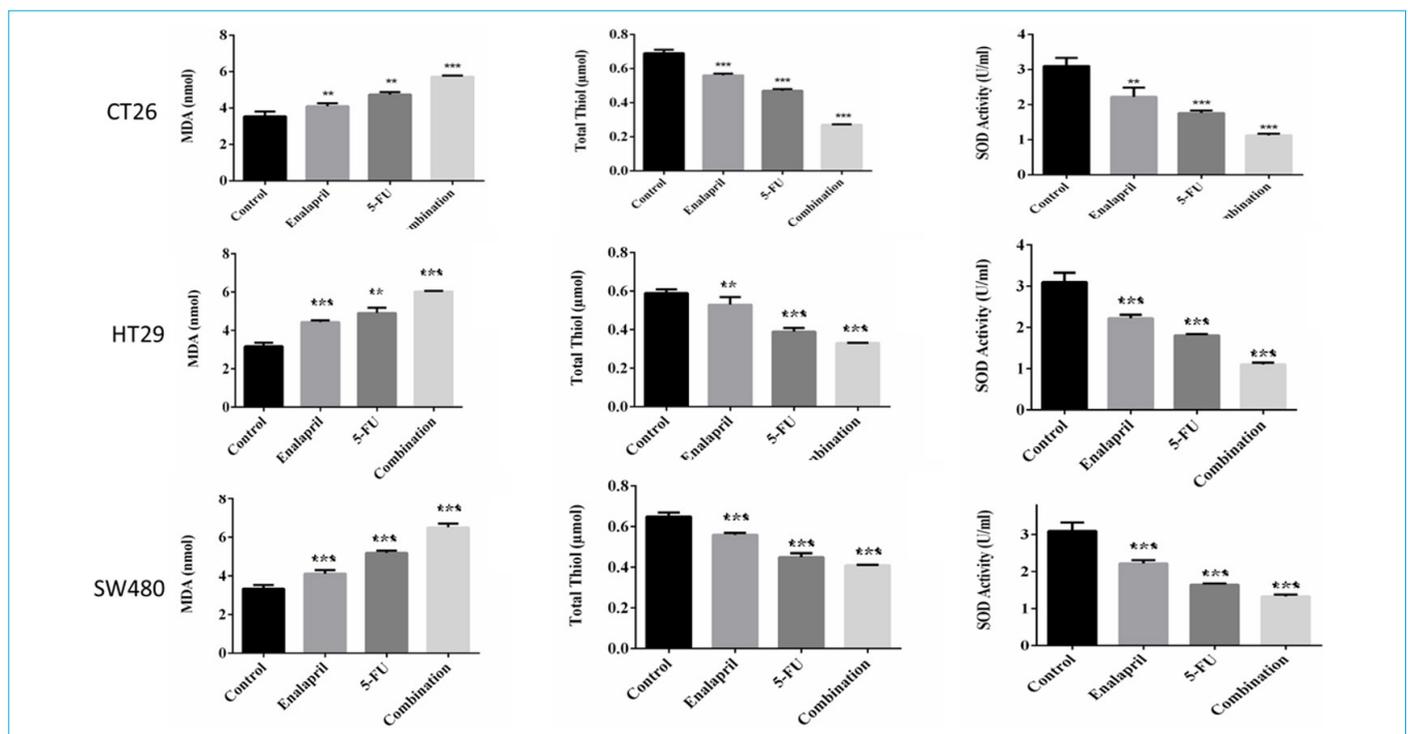
**Figure 2.** The apoptotic activity of agents; **(a)** DAPI staining displayed DNA fragmentation and nuclear apoptosis and confirmed apoptotic cell death in  $IC_{50}$  concentration in CRC treated cells, **(b)** the level of caspase activity.



**Figure 3.** Induces apoptosis of CRC cells; CT-26 cells were treated with enalapril for 24 h and apoptosis was explored by flow cytometry using annexin V/PI staining. The values of the lower right and the upper right area indicate the percentage of the cells in early and late apoptosis, respectively.



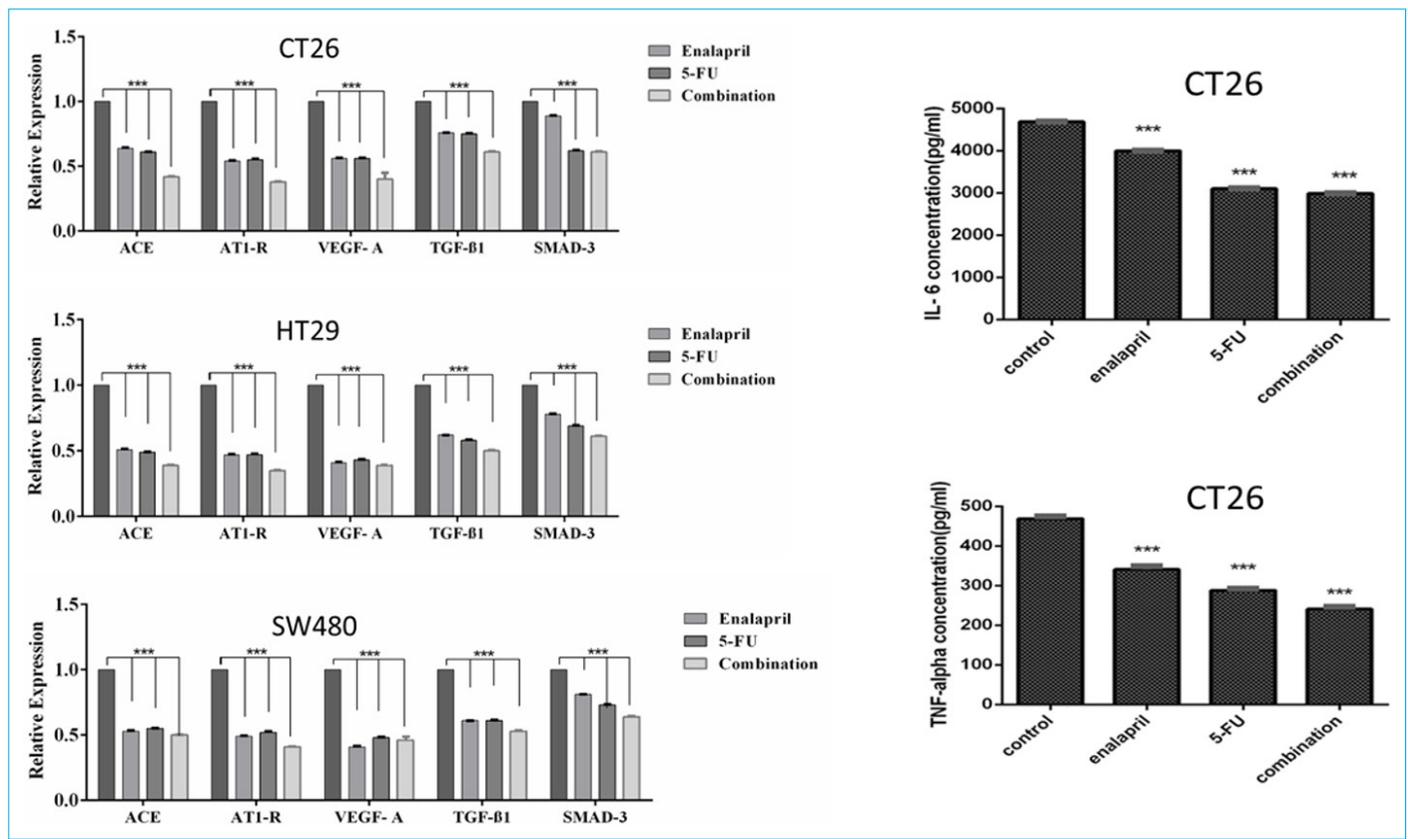
**Figure 4.** Modulation of oxidant and antioxidant activities; **(a, b)** the effects of enalapril and 5-FU on production of cellular ROS were explored in CRC cells (\*\* $p < 0.001$  compared to control group).



**Figure 5.** Regulatory effects of enalapril and 5-FU on MDA, SOD and thiol levels in CRC cells (\*\* $p < 0.001$  compared to control group).

inflammatory responses by regulating the oxidative stress of CRC cells. It can be concluded that a significant reduction was observed in the levels of ACE, AT1-R, TGF- $\beta$ , and

SMAD3 throughout the cells that were treated with enalapril, which was indicative of the inhibition of ACE/ARB pathway in CRC cells (Fig. 6a).



**Figure 6.** (a) The expression level of ACE, AT1-R, TGF-beta1 VEGF-A and Smad-3 genes in CRC cells treated with enalapril and 5-FU as detected by real-time RT-PCR, (b) Enalapril decrease cytokine expression of TNF- $\alpha$ , (c) IL-6 in CT-26 cells (\*\* $p < 0.001$  compared to control group).

## Discussion

The anti-proliferative property of enalapril and its interaction with 5-FU in CRC was demonstrated in this work for the first time. It was indicated by our data that targeting angiotensin II type I pathway could result in inhibition in the cell growth in colorectal cancer, which could be achieved by restraining the TGF-beta pathway and inflammatory markers (Fig. 4d). According to the observations, the functionality of RAS system in cancer is so crucial and significant that the Ang II-AT1R system has recently gained such substantial attention as a therapeutic option in the treatment of different malignancies, including CRC.<sup>[10]</sup> Ang II is capable of modulating PI3K/Akt pathways.<sup>[14]</sup> We exhibited in this work that the expression of AT1-R and ACE could be decreased through the application of enalapril, which could also increase the caspase-9/3 activation. Furthermore, Ang II is able to minimize cell adhesion/invasion via type 1 receptor, which is caused by the induced reduction in the expression of integrins.<sup>[12]</sup> Ang II has been reported to take action through AT1Rs and increases the expression of VEGF, which leads to the occurrence of cell growth, migration, and angiogenesis.<sup>[13]</sup> Relative observations have stated that the activation of AT1R can perturbate cytokines via MAP kinase, ROS

activation, and NF- $\kappa$ B pathways.<sup>[18]</sup> It has been reported that ACE-I can suppress cell growth and angiogenesis<sup>[19]</sup> through the RAS-dependent inhibition of Ang II levels.<sup>[5]</sup> The Intrinsic metal-chelating properties of certain ACE-I could be assumed as the reason behind their anti-cancer functionality, since this substance is responsible for the suppression of MMPs.<sup>[20]</sup> In addition, ACE-I can directly decrease the plasminogen activator inhibitor and sulfhydryl group, which leads to the inhibition of angiogenesis.<sup>[21]</sup> It is also notable that the depletion of ROS could activate MMPs and VEGF.

A few studies have described that ACE-I may actually encourage cell growth due to their immune-modulatory functionality and may increase the rate of kidney cancer.<sup>[22]</sup> A study on 5027 patients has suggested that ACE-I may be effective in post-anthracycline cardiotoxicity throughout the treatment of pediatric cancer.<sup>[23]</sup> However, several studies have shown that genetic variant and alteration in ACE and ARB pathways are associated with the increased risk of developing different malignancies, which further points out the significant value of this pathway as a potential target. Our results showed that enalapril or 5-FU alone cannot significantly contribute to the improvement of CRC while the two drugs synergistically exacerbate the

above effects. Because tumor invasion and therapeutic resistance are closely related to EMT, NF- $\kappa$ B/STAT3 and -TGF- $\beta$  signaling are essential for the promotion of EMT in colorectal cancer.

We investigated the effect of the combination of two drugs on cell migration and E-cadherin expression and the results showed that the combination of enalapril and 5-FU significantly reduced cell migration. In summary, this work exhibited the therapeutic impact of enalapril and its combination with a standard chemotherapeutic agent (5-FU) in the treatment of CRC for the first time, nevertheless, further *in vivo* and clinical trial studies are required to explore the value of these strategies for being applied in the treatment of this disease.

#### Disclosures

**Ethics Committee Approval:** The study was approved by the Local Ethics Committee.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

#### References

1. Hashemzahi M, Naghibzadeh N, Asgharzadeh F, Mostafapour A, Hassanian SM, Ferns GA, et al. The therapeutic potential of losartan in lung metastasis of colorectal cancer. *EXCLI J* 2020;19:927–35.
2. Stoeltzing O, Liu W, Reinmuth N, Parikh A, Ahmad SA, Jung YD, et al. New approaches to the treatment of hepatic malignancies angiogenesis and antiangiogenic therapy of colon cancer liver metastasis. *Ann S Oncol* 2003;10:722–33.
3. Moses HL, Yang EY, Pietenpol JA. TGF- $\beta$  stimulation and inhibition of cell proliferation: New mechanistic insights. *Cell* 1990;63:245–7.
4. Massagué J. G1 cell-cycle control and cancer. *Nature* 2004;432:298–306.
5. Lindberg H, Nielsen D, Jensen BV, Eriksen J, Skovsgaard T. Angiotensin converting enzyme inhibitors for cancer treatment? *Acta Oncol* 2004;43:142–52.
6. Kerbel RS. Clinical trials of antiangiogenic drugs: Opportunities, problems, and assessment of initial results. *J Clin Oncol* 2001;19:45–51.
7. Kim SI, Kwak JH, Na H-J, Kim JK, Ding Y, Choi ME. Transforming growth factor- $\beta$  (TGF- $\beta$ 1) activates TAK1 via TAB1-mediated autophosphorylation, independent of TGF- $\beta$  receptor kinase activity in mesangial cells. *J Biol Chem* 2009;284:22285–96.
8. Ludwig L, Kessler H, Wagner M, Hoang-Vu C, Dralle H, Adler G, et al. Nuclear factor- $\kappa$ B is constitutively active in C-cell carcinoma and required for RET-induced transformation. *Cancer Res* 2001;61:4526–35.
9. Strippoli GF, Bonifati C, Craig ME, Navaneethan SD, Craig JC. Angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists for preventing the progression of diabetic kidney disease. *Cochrane Database Syst Rev* 2006;2006:006257.
10. Du N, Feng J, Hu L-J, Sun X, Sun H-B, Zhao Y, et al. Angiotensin II receptor type 1 blockers suppress the cell proliferation effects of angiotensin II in breast cancer cells by inhibiting AT1R signaling. *Oncol Rep* 2012;27:1893–903.
11. Osumi H, Matsusaka S, Wakatsuki T, Suenaga M, Shinozaki E, Mizunuma N. Angiotensin II type-1 receptor blockers enhance the effects of bevacizumab-based chemotherapy in metastatic colorectal cancer patients. *Mol Clin Oncol* 2015;3:1295–300.
12. Puddefoot J, Udeozo U, Barker S, Vinson G. The role of angiotensin II in the regulation of breast cancer cell adhesion and invasion. *Endocr Relat Cancer* 2006;13:895–903.
13. Ribatti D, Conconi MT, Nussdorfer GG. Nonclassic endogenous novel regulators of angiogenesis. *Pharmacol Rev* 2007;59:185–205.
14. Zhao Y, Chen X, Cai L, Yang Y, Sui G, Wu J. Angiotensin II suppresses adriamycin-induced apoptosis through activation of phosphatidylinositol 3-kinase/Akt signaling in human breast cancer cells. *Acta Biochim Biophys Sin* 2008;40:304–10.
15. Papageorgis P, Cheng K, Ozturk S, Gong Y, Lambert AW, Abdolmaleky HM, et al. Smad4 inactivation promotes malignancy and drug resistance of colon cancer. *Cancer Res* 2011;71:998–1008.
16. Samuel S, Fan F, Dang LH, Xia L, Gaur P, Ellis LM. Intracrine vascular endothelial growth factor signaling in survival and chemoresistance of human colorectal cancer cells. *Oncogene* 2011;30:1205–12.
17. Rivera E, Arrieta O, Guevara P, Duarte-Rojo A, Sotelo J. AT1 receptor is present in glioma cells; its blockage reduces the growth of rat glioma. *Br J Cancer* 2001;85:1396–9.
18. El Bekay R, Alvarez M, Monteseirín J, Alba G, Chacón P, Vega A, et al. Oxidative stress is a critical mediator of the angiotensin II signal in human neutrophils: Involvement of mitogen-activated protein kinase, calcineurin, and the transcription factor NF- $\kappa$ B. *Blood* 2003;102:662–71.
19. Yoshiji H, Kuriyama S, Kawata M, Yoshii J, Ikenaka Y, Noguchi R, et al. The angiotensin-I-converting enzyme inhibitor perindopril suppresses tumor growth and angiogenesis: Possible role of the vascular endothelial growth factor. *Clin Cancer Res* 2001;7:1073–8.
20. Williams R, Parsons S, Morris T, Rowlands B, Watson S. Inhibition of matrix metalloproteinase activity and growth of gastric adenocarcinoma cells by an angiotensin converting enzyme inhibitor in *in vitro* and murine models. *Eur J Surg Oncol* 2005;31:1042–50.

21. de Groot-Besseling RR, Ruers TJ, van Kraats AA, Poelen GJ, Ruiten DJ, de Waal RM, et al. Anti-tumor activity of a combination of plasminogen activator and captopril in a human melanoma xenograft model. *Int J Cancer* 2004;112:329–34.
22. Wysocki PJ, Kwiatkowska EP, Kazimierzak U, Suchorska W, Kowalczyk DW, Mackiewicz A. Captopril, an angiotensin-converting enzyme inhibitor, promotes growth of immunogenic tumors in mice. *Clin Cancer Res* 2006;12:4095–102.
23. Silber JH, Cnaan A, Clark BJ, Paridon SM, Chin AJ, Rychik J, et al. Enalapril to prevent cardiac function decline in long-term survivors of pediatric cancer exposed to anthracyclines. *J Clin Oncol* 2004;22:820–8.