The *In Vivo* Radiosensitizing Effect of Magnolol on Tumor Growth of Hepatocellular Carcinoma

YU-SHAN CHEN[1#], ROU SUN[2#], WEI-LUNG CHEN[3,4#], YU-CHEN YAU[5#], FEI-TING HSU[6], JING-GUNG CHUNG[6], CHIA-JUNG TSAI[7], CHIA-LING HSIEH[8,9*], YING-MING CHIU[10,11*] and JIANN-HWA CHEN[12,13*]

[1]*Department of Radiation Oncology, Show Chwan Memorial Hospital, Changhua, Taiwan, R.O.C.;*
[2]*Department of Medical Imaging, Taipei Medical University Hospital, Taipei, Taiwan, R.O.C.;*
[3]*Department of Emergency Medicine, Cathay General Hospital, Taipei, Taiwan, R.O.C.;*
[4]*School of Medicine, Fu Jen Catholic University, Taipei, Taiwan, R.O.C.;*
[5]*Department of Radiation Oncology, Chang Bing Show Chwan Memorial Hospital, Lukang, Taiwan, R.O.C.;*
[6]*Department of Biological Science and Technology, China Medical University, Taichung, Taiwan, R.O.C.;*
[7]*Department of Medical Imaging and Radiological Sciences, I-Shou University, Kaohsiung, Taiwan, R.O.C.;*
[8]*The Ph.D. Program for Translational Medicine, College of Medical Science and Technology,*
Taipei Medical University, Taipei, Taiwan, R.O.C.;
[9]*TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei, Taiwan, R.O.C.;*
[10]*Rheumatology and Immunology Center, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[11]*School of Medicine, China Medical University, Taichung, Taiwan, R.O.C.;*
[12]*Department of Emergency Medicine, Cathay General Hospital, Taipei, Taiwan, R.O.C.;*
[13]*School of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan, R.O.C.*

Abstract. *Background/Aim: Radiation (RT) induced ERK/NF-κB in hepatocellular carcinoma (HCC) has been reported in our previous works; it weakens the toxicity of RT or triggers a radioresistance effect. Thus, combining RT with a suitable NF-κB inhibitor may sensitize HCC to RT. Magnolol, a bioactive compound, was known to have anti-inflammatory and anti-tumor functions. Here, we aimed to investigate whether magnolol may enhance anti-HCC efficacy of RT in vivo. Materials and Methods: We established a Hep3B bearing mouse to evaluate the efficacy of the combination treatment of magnolol and RT. Results: Most significantly, tumor volume and tumor weight inhibition was found in the combination group. Tumor immunohistochemistry staining also illustrated the suppression of RT-induced ERK/NF-κB-related proteins expression by magnolol. In addition, intrinsic apoptosis-related proteins, such as caspase-3 and -9, were markedly increased in the combination group. Conclusion: Magnolol may effectively enhance anti-HCC ability of RT by downregulating the expression of ERK/NF-κB-related proteins and increasing the expression of apoptosis-related proteins.*

This article is freely accessible online.

[#,*]These Authors contributed equally to this study.

Correspondence to: Chia-Ling Hsieh, Associate Professor, The Ph.D. Program for Translational Medicine, College of Medical Science and Technology, Taipei Medical University, TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei 110, Taiwan, R.O.C. Tel: +886 0227361661 ext.7635, e-mail: chsieh2@tmu.edu.tw; Ying-Ming Chiu, MD, Rheumatology and Immunology Center, China Medical University Hospital, School of Medicine, China Medical University, Taichung, Taiwan, R.O.C. Tel: +886 422053366 ext. 2502, e-mail: ymcgreen@yahoo.com.tw; D7028@mail.cmuh.org.tw; Jiann-Hwa Chen, MD, Department of Emergency Medicine, Cathay General Hospital, School of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan, R.O.C. Tel: +886 953661292, e-mail: chenjiannhwa@yahoo.com.tw; cgh08335@cgh.org.tw

Key Words: Magnolol, radiation, hepatocellular carcinoma, apoptosis.

Hepatocellular carcinoma (HCC), the most common type of liver cancer in adults, ranks as the fourth leading cause of cancer-related mortality worldwide (1). Patients with advanced HCC are unsuitable candidates for curative treatment options including surgery, liver transplantation, and percutaneous ablation (2). The low tolerance of whole liver to radiation has restricted application of radiotherapy (RT) for HCC in the past. Recent technology advancements in RT such as intensity-modulated RT (IMRT), image-guided RT (IGRT), hypo-fractionated radiotherapy (HFRT), and stereotactic body RT (SBRT) can precisely deliver high-dose radiation to HCC while reducing noncancerous liver tissue irradiation (3-5). RT
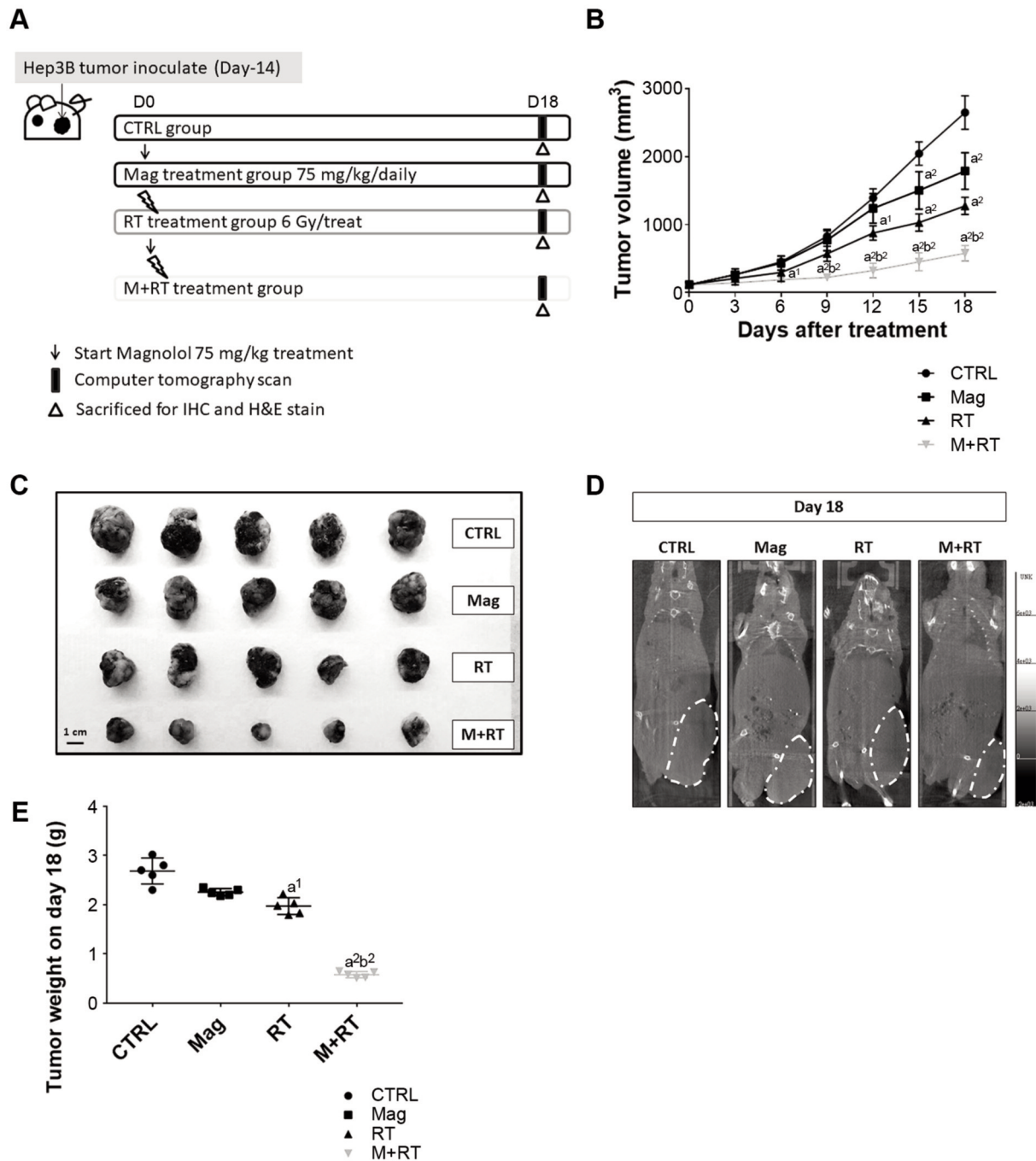
Figure 1. *Tumor growth is suppressed by the combination of magnolol with RT in a Hep3B tumor bearing model. (A) The experimental flow chart of the in vivo study. (B) Tumor growth trend was recorded by calliper every three days. (C) The extracted tumors on day 18 are photographed and are displayed. (D) Whole-body CT image from each treatment group on day 18. (E) The weight of the extracted tumors is measured and quantified. ($a^1 p<0.05$ and $a^2 p<0.01$ vs. CTRL; $b^1 p<0.05$ and $b^2 p<0.01$ vs. single treatment)*

has been shown to provide excellent local control and palliative efficacy in unresectable or metastatic HCC (6).

In addition to RT technology, radiosensitizers that sensitize HCC to radiation have been developing. Natural products such as ellagic acid, curcumin, and mesima have been indicated as potential radiosensitizers to boost anti-HCC efficacy of radiation. Induction of mitochondrial apoptosis, arrest of cell cycle G_2/M phase, and inhibition of
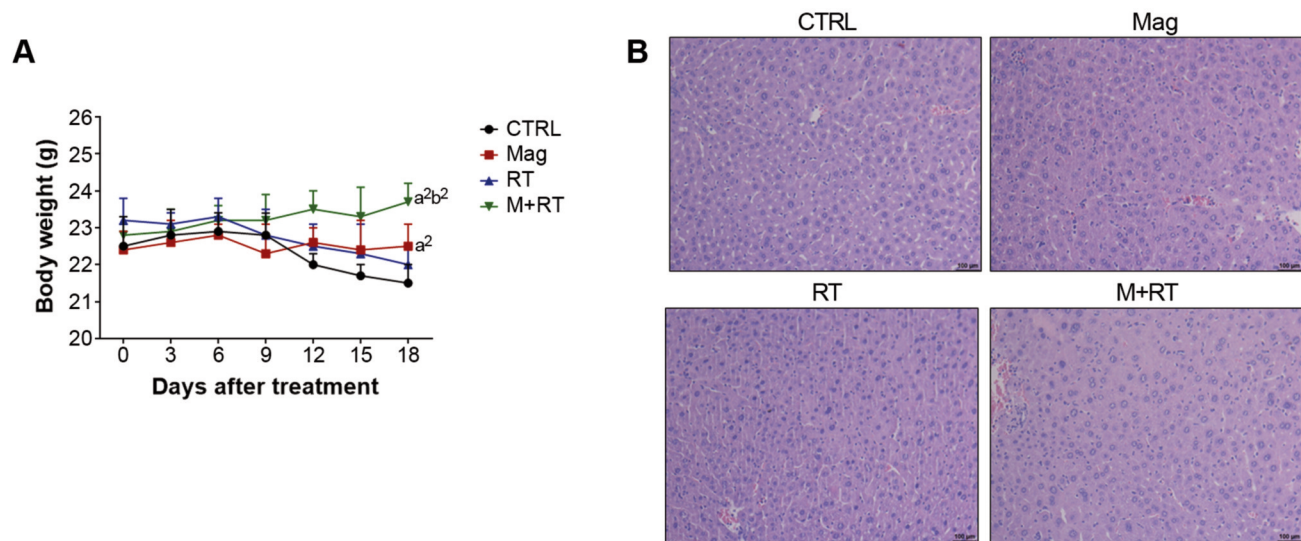
Figure 2. *No general toxicity is observed following treatment with the combination of magnolol with RT in Hep3B tumor bearing model. (A) Body weight of each mouse was recorded every three days. (B) Representative liver H&E staining results from each group.* (a^2 *p<0.01 vs. CTRL;* b^2 *p<0.01 vs. single treatment*)

NF-κB activation have been associated with the radiosensitizing effect of natural products in HCC (7-9). Magnolol, one of major components of *Magnolia officinalis*, has been shown to trigger apoptosis through death receptor (extrinsic)/mitochondrial (intrinsic) pathways and to down-regulate extracellular signal-regulated kinase (ERK)/NF-κB signaling in HCC *in vitro* and *in vivo* (10, 11). Magnolol was also recognized to have an anti-inflammation and anti-tumor potential (12). However, whether magnolol functions as radiosensitizer and enhances anti-HCC efficacy of radiation has not yet been elucidated. Therefore, the main purpose of the present study was to investigate the therapeutic efficacy and possible underlying mechanism of magnolol combined with radiation in HCC *in vivo*.

Materials and Methods

Cell lines. Hep3B cells were purchased from Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium), containing with 10% fetal bovine serum, 1% penicillin and streptomycin and 2 mM L-glutamine (Thermo Fisher Scientific, Fremont, CA, USA) at 37˚C in 5% CO_2 humidified atmosphere.

Animal experiment. All animal experiments were performed in accordance with the protocols approved by the Animal Care and Use Committee at China Medical University (approval number: CMU IACUC-2019-206). Six-week-old male BALB/cAnN.Cg-Foxn1nu/CrlNarl nude mice were purchased from the National Laboratory Animal Centre (Taipei, Taiwan) and housed in a pathogen-free animal facility. The animals were fed sterilised mouse chow and water. Mice (20-25 g) were subcutaneously injected with Hep3B cells ($5×10^6$) on the right leg. When tumor volume reached 80 mm^3, mice were randomly separated into 4 groups (n=16), including CTRL group (0.1% DMSO daily gavage treatment), Mag group (75 mg/kg magnolol daily gavage treatment), RT group (once dosage of 6 Gy radiotherapy), and combination therapy. All treatments were initiated at day 0, which is indicated in Figure 1A. Tumor volume was measured every three days and calculated using the following formula: Volume=Height×$Weight^2$×0.523. Computer tomography for tumor size assessment was performed on day 18. Mice were sacrificed on day 18 for further liver pathology and tumor protein expression evaluation. All animal experiments were repeated twice.

Radiation exposure. After anesthesia, mice were irradiated by using clinical linear accelerator (6 MV photons, Elekta Synergy linear accelerator, Crawley, UK). Prescribed dose to gross tumor volume was 6 Gy and 1.0 cm bolus was used to cover tumor for dose build up.

Computer tomography (CT) scanning. After eighteen days of treatment, mice were assessed with CT scan (Mediso Ltd., Budapest, Hungary) to evaluate their tumor size. Hep3B bearing animals were sedated by 1-3% isoflurane during whole-body CT examination. The scanning parameters were as follow: tube energy=55 kVp×145 μA; direction=360˚; Voxel size=145×145×145 μM (13).

Immunohistochemistry (IHC) staining. After eighteen days of treatment, mice were sacrificed and tumor tissues were extracted for IHC stain. Formalin-fixed (4%), paraffin-embedded tissues were cut into 5 μm sections and subjected to IHC staining, as described previously (14). The sections were immunohistochemically stained using antibodies against cleaved-caspase-3 (E-AB-30004, Elabscience, Houston, TX, USA), cleaved-caspase-8 (E-AB-22107, Elabscience), cleaved-caspase-9 (10380-1-AP, Proteintech, Rosemont, IL, USA), Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (E-AB-
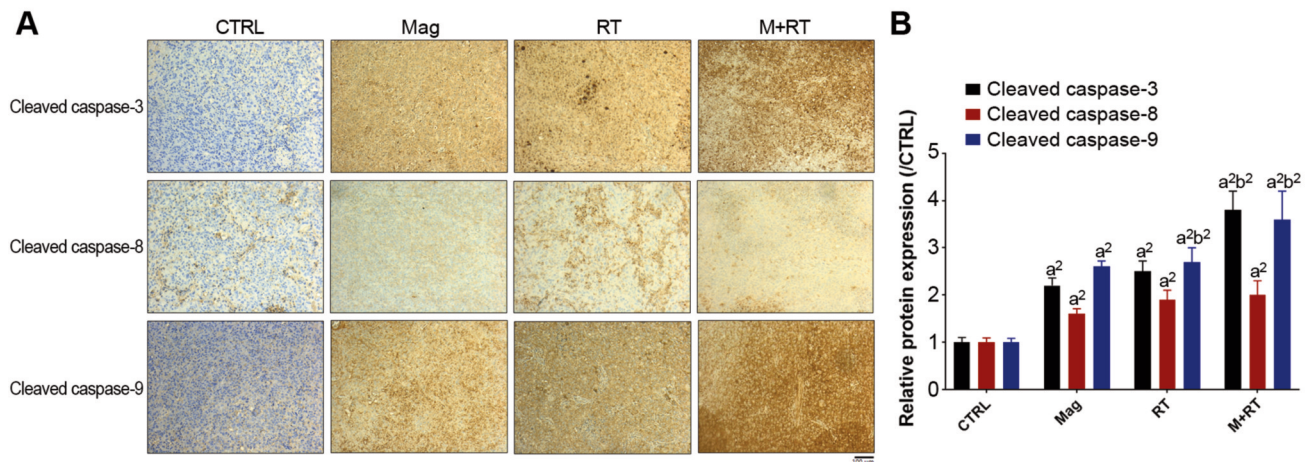
Figure 3. *Intrinsic apoptosis related proteins are enhanced by the combination treatment with magnolol and RT in Hep3B tumor bearing model. (A) Representative tumor IHC staining results of cleaved-caspase-3, -8 and -9 from each group is displayed. (B) Quantification of the results of each group is presented as a bar chart. (a^2 p<0.01 vs. CTRL; b^2 p<0.01 vs. single treatment) Scale bars: 100 μm.*

21303, Elabscience), Phospho-NF-κB p65 (Ser276) (#3037, Cell Signaling Technology, Danvers, MA, USA), Ki-67 (E-AB-63523, Elabscience), C-FLIP (E-AB-16437, Elabscience), and MCL-1 (E-AB-33430, Elabscience). IHC stained sections were then imaged by microscope (Nikon ECLIPSE Ti-U, Tokyo, Japan) at 100´ magnification. Finally, the positive staining result of each protein was quantified by ImageJ software version 1.50 (National Institutes of Health, Bethesda, MD, USA).

Hematoxylin and eosin (H&E) staining. Liver tissue extracted from mice on day 18 was prepared for H&E staining. The fixation, paraffin-embedding and slicing procedure was the same as for IHC staining. Hematoxylin and eosin (H&E) staining was executed by Bio-Check Laboratories Ltd (New Taipei, Taiwan) as previously described (15). H&E stained sections were also photographed by Nikon microscope at 100´ magnification.

Statistical analysis. Excel 2017 was used to analyse data. All data are presented as mean±SD. Statistical significance was evaluated through the conventional Student's *t*-test and one-way ANOVA. *p*<0.05 was regarded as statistically significant.

Results

Magnolol markedly enhanced the anti-HCC growth effect of RT. To identify whether magnolol may increase the therapeutic efficacy of RT, we established a Hep3B tumor bearing animal model. The experimental flow chart is displayed in Figure 1A. Mice were randomly separated into four groups, including CTRL, MAG (magnolol 75 mg/kg), RT (6 Gy) and magnolol combined with RT (M+RT), when mice tumor reached 80 mm^3. Tumor volume and tumor size were recorded every three days. As shown in Figure 1B, the slowest tumor growth trend was shown in the M+RT group. Tumors extracted from the mice on the final day of therapy

were also photographed and were displayed in Figure 1C. The smallest tumor size was found in the M+RT group, which corresponded to the tumor volume results. Furthermore, mice whole-body CT images were taken before sacrifice. Notable tumor growth inhibition was observed in the M+RT group compared to single treatment (Figure 1D). Finally, we also measured the weight of the extracted tumors. The lightest tumor was presented in the M+RT group compared to all single therapies (Figure 1E). Taken together, the above results indicated that magnolol effectively sensitized HCC to RT.

Mice body weight and liver pathology were not affected by magnolol combined with RT in HCC bearing mice. To investigate whether the combination of magnolol with RT may induce general toxicity in mice, we recorded mouse body weight and extracted liver for H&E staining. As shown in Figure 2A, both the M+RT and the magnolol single treatment groups of mice maintained their body weight during the whole treatment process. The non-treated control and the RT group showed marked body weight loss compared to M+RT. In addition, we performed H&E staining on mice liver to investigate liver pathology after treatment. There was no obvious change on liver pathology following the different therapies (Figure 2B). In conclusion, magnolol and RT combination therapy may not induce general toxicity in Hep3B bearing mice.

Magnolol markedly enhanced RT-induced intrinsic apoptosis signalling in HCC bearing mice. Next, we investigated whether apoptosis related mechanisms were affected by the combination of magnolol with RT using IHC staining of the mouse tumor tissue. As indicated in Figure 3A and B, protein expression of the apoptosis marker cleaved caspase-3 was markedly increased 1 to 1.5 times by M+RT compared to
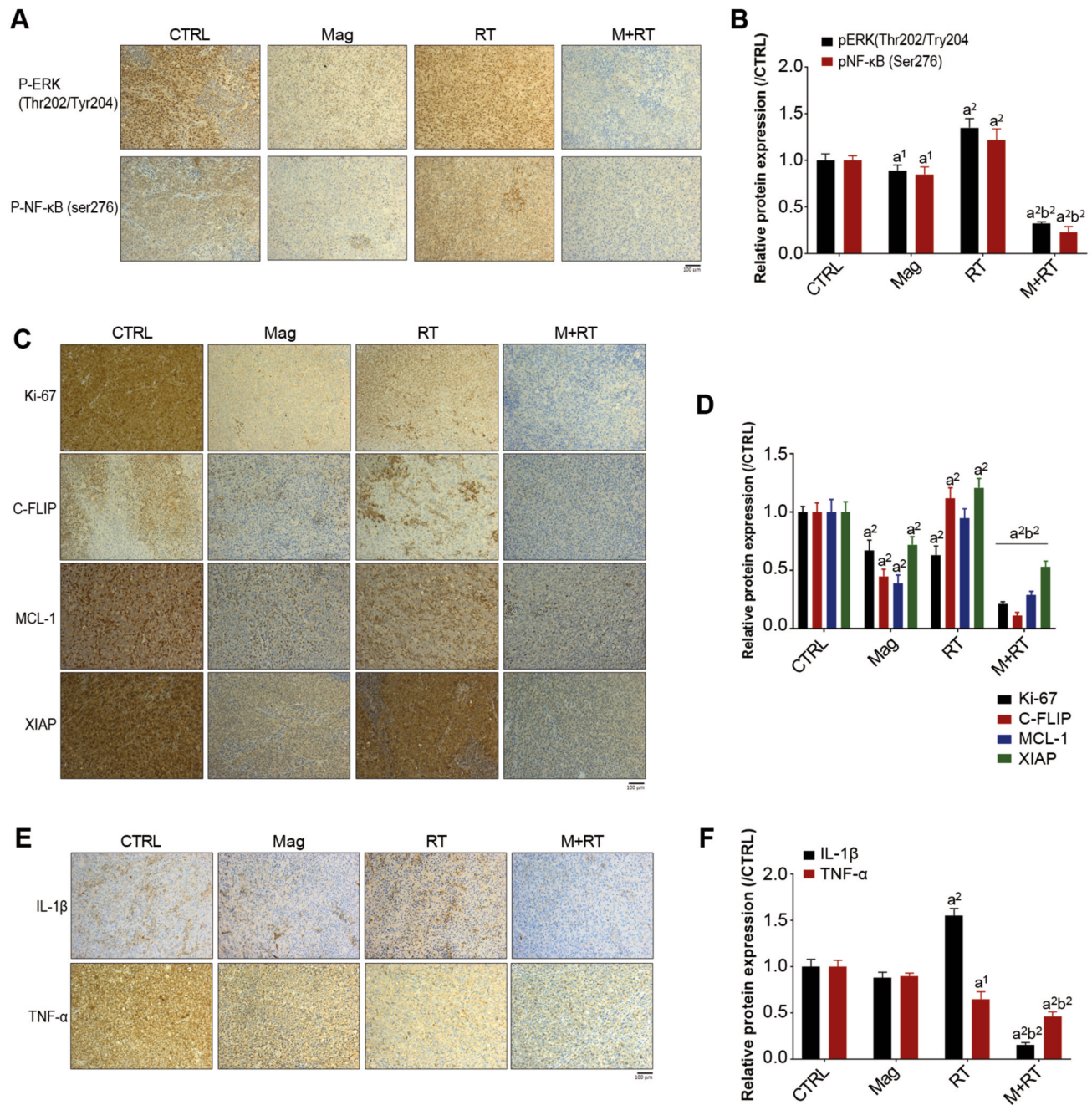
Figure 4. *RT induced phosphorylation of ERK/NF-κB and expression of their downstream proteins was blocked by magnolol in Hep3B tumor bearing model. (A, C, E) Representative tumor IHC staining results of P-ERK, P-NF-κB, Ki-67, C-FLIP, MCL-1, XIAP, IL-1β and TNF-α from each group. (B, D, F) Quantification of the results of each group is shown as bar chart. (a[1] p<0.05 and a[2] p<0.01 vs. CTRL; b[2] p<0.01 vs. single treatment) Scale bars: 100 μm.*

single treatments. Furthermore, protein expression of cleaved caspase-9, a recognized intrinsic apoptosis marker, was also augmented by M+RT compared to the single treatment. Similar induction of cleaved caspase-8 protein, an extrinsic apoptosis marker, was found in all treatment groups. Taken

together, magnolol may effectively enhance the induction of intrinsic apoptosis by RT.

Magnolol diminished RT-activated ERK/NF-κB and its downstream signalling in HCC bearing mice. A previous study
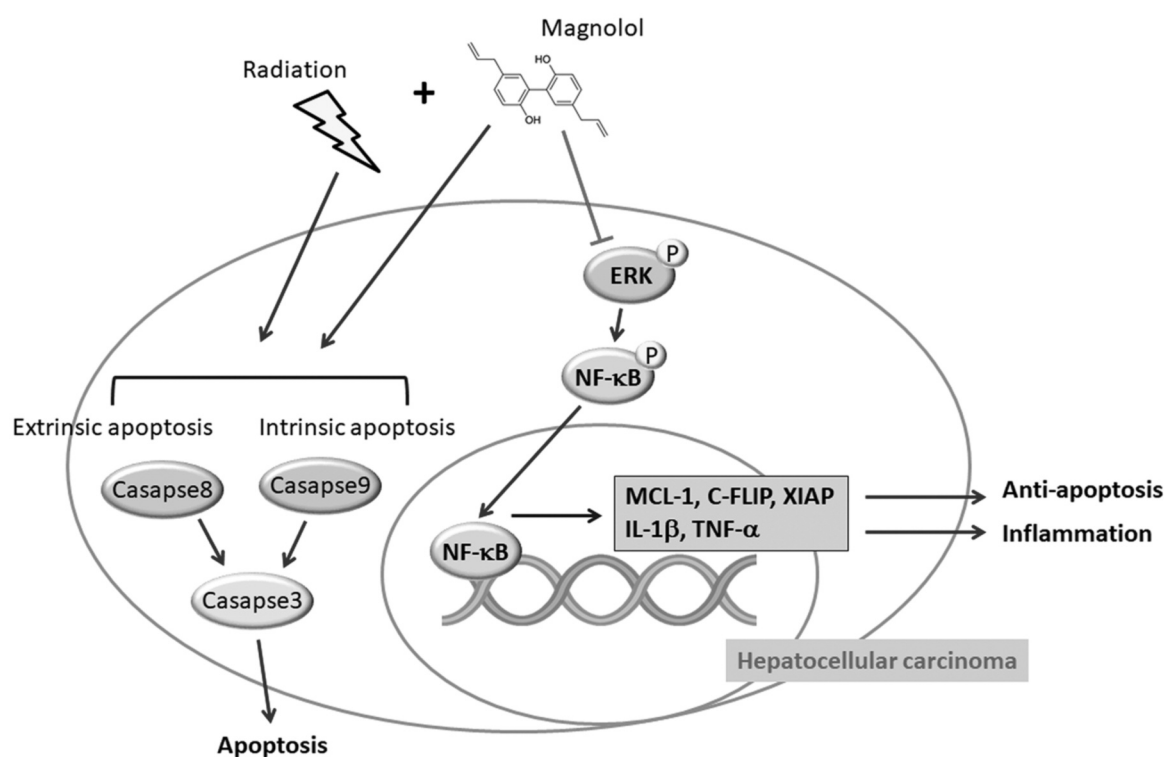
Figure 5. *The proposed mechanism of the effect of the combination of magnolol with RT. Arrow indicates induction. Magnolol may trigger the apoptosis pathway and suppress ERK/NF-κB related signal transduction.*

has suggested that RT may activate ERK/NF-κB and expression of their downstream proteins in HCC (16). Here, we aimed to evaluate whether magnolol may suppress RT-induced ERK/NF-κB mediated signal transduction. As illustrated in Figure 4A and B, RT-induced expression of phospho-ERK and phospho-NF-κB were diminished by combination of RT with magnolol. The proliferation marker, Ki-67 was effectively reduced in the M+RT group compared to single treatment (Figure 4C and D). Furthermore, NF-κB mediated expression of anti-apoptosis proteins including C-FLIP, MCL-1 and XIAP was markedly reduced by M+RT treatment. NF-κB-mediated expression of inflammation proteins such as IL-1β and TNF-α were also markedly suppressed by the combination of magnolol with RT (Figure 4E and F). These results indicated that magnolol may effectively suppress RT-induced ERK/NF-κB pathway and the expression of the related anti-apoptosis and inflammation proteins.

Magnolol enhanced RT efficacy via the induction of apoptosis signalling and inhibition of ERK/NF-κB mediated pathways in HCC bearing mice. As showed in Figure 5, both RT and magnolol may induce the expression of the extrinsic and intrinsic apoptosis-related proteins, included cleaved-caspase-3, -8 and -9. In addition, the ERK/NF-κB signalling transduction that induced by RT can be mitigated by

magnolol. The NF-κB downstream signalling that is involved in anti-apoptosis and inflammation was decreased by magnolol. In summary, magnolol may facilitate RT's anti-HCC potential through triggering apoptosis and inhibiting ERK/NF-κB pathway.

Discussion

In our study, the therapeutic efficacy of the combination of magnolol with radiation (high-energy x-rays) was evaluated by using HCC bearing mice. We found that magnolol was a radiosensitizer that significantly promoted radiation-mediated inhibition of tumor growth in HCC *in vivo*. Cleaved-caspase-3, -8, and -9 are required for radiation-induced apoptosis through the extrinsic and intrinsic pathways (17). Our findings showed that the combination of magnolol and radiation significantly increased expression of both cleaved-caspase-3 and caspase-9 compared to magnolol or radiation alone treatment (Figure 3). Magnolol may enhance radiation-induced apoptosis through the intrinsic apoptotic pathway in HCC.

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is the key transcription factor which mediates cancer cell radioresistance by regulating the expression of anti-apoptotic genes. Anti-apoptotic proteins C-FLIP, MCL-1, and XIAP limit the anti-cancer efficacy of radiation by inhibiting

the extrinsic and intrinsic apoptosis signaling transduction (18, 19). In a previous study, inhibitor of κBα mutant vector(p-IκBαM), a super repressor of NF-κB, not only augmented radiation-induced cytotoxicity but also inhibited expression of radiation-triggered NF-κB-related proteins in HCC (16). The increased expression of proinflammatory cytokines TNF-α and IL-1β as unfavorable prognostic markers was associated with recurrence of HCC (20). QNZ, an inhibitor of NF-κB signaling, has been shown to decrease protein levels of C-FLIP, MCL-1, XIAP, TNF-α, and IL-1 β in HCC *in vitro* (21, 22). Our data demonstrated that magnolol promoted radiation-decreased phosphorylation of NF-κB, protein levels of C-FLIP, MCL-1, XIAP, TNF-α, and IL-1 β in HCC *ex vivo* (Figure 4).

ERK, a member of activated protein kinase (MAPK) family, is the downstream component of RAF/mitogen-activated protein/ERK kinase (MEK)/ERK signaling pathway involved in tumor progression (23). ERK has been demonstrated to regulate expression of proliferation, anti-apoptotic, and invasion-related proteins through activation of the NF-κB signaling in HCC (10, 20). Furthermore, radioresistance of cancer cells has been linked to the phosphorylation of ERK (24). Sorafenib, an inhibitor of RAF/MEK/ERK signaling, has been shown to reverse the radiation-induced NF-κB activity and expression of NF-κB-related proteins in HCC (16). Magnolol has been shown to reduce NF-κB activation and expression of NF-κB-related proteins through upregulating dephosphorylation of ERK in HCC *in vitro* and *in vivo* (10, 11). In this study, magnolol inhibited radiation-induced ERK phosphorylation in HCC *in vivo* (Figure 4A).

In conclusion, magnolol is a radiosensitizer that enhances the therapeutic effect of radiation in HCC *in vivo*. The radiosensitizing effect of magnolol on tumor growth of hepatocellular carcinoma *in vivo* is mediated through the induction of the intrinsic apoptosis pathway and suppression of NF-κB signaling.

Conflicts of Interest

The Authors declare no competing financial interests regarding this study.

Authors' Contributions

YSC, RS, WLC and YCY performed the experiments. FTH, JGC and CJT prepared the initial version of the paper. CLH, YMC and JHC designed the study, performed the literature review, and prepared the final versions of the paper.

Acknowledgements

References

1 Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A and Roberts LR: A global view of hepatocellular carcinoma: Trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol *16(10)*: 589-604, 2019. PMID: 31439937. DOI: 10.1038/s41575-019-0186-y

2 Forner A, Hessheimer AJ, Isabel Real M and Bruix J: Treatment of hepatocellular carcinoma. Crit Rev Oncol Hematol *60(2)*: 89-98, 2006. PMID: 16860993. DOI: 10.1016/j.critrevonc.2006.06.001

3 Nakajima T, Ninomiya Y and Nenoi M: Radiation-induced reactions in the liver - modulation of radiation effects by lifestyle-related factors. Int J Mol Sci *19(12)*: 3855, 2018. PMID: 30513990. DOI: 10.3390/ijms19123855

4 Wu CH, Chen CY, Yeh CT and Lin KH: Radiosensitization of hepatocellular carcinoma through targeting radio-associated microrna. Int J Mol Sci *21(5)*, 2020. PMID: 32182776. DOI: 10.3390/ijms21051859

5 Hara K, Takeda A, Tsurugai Y, Saigusa Y, Sanuki N, Eriguchi T, Maeda S, Tanaka K and Numata K: Radiotherapy for hepatocellular carcinoma results in comparable survival to radiofrequency ablation: A propensity score analysis. Hepatology *69(6)*: 2533-2545, 2019. PMID: 30805950. DOI: 10.1002/hep.30591

6 Chen CP: Role of radiotherapy in the treatment of hepatocellular carcinoma. J Clin Transl Hepatol *7(2)*: 183-190, 2019. PMID: 31293919. DOI: 10.14218/JCTH.2018.00060

7 Das U, Biswas S, Chattopadhyay S, Chakraborty A, Dey Sharma R, Banerji A and Dey S: Radiosensitizing effect of ellagic acid on growth of hepatocellular carcinoma cells: An *in vitro* study. Sci Rep *7(1)*: 14043, 2017. PMID: 29070894. DOI: 10.1038/s41598-017-14211-4

8 Hsu FT, Liu YC, Liu TT and Hwang JJ: Curcumin sensitizes hepatocellular carcinoma cells to radiation *via* suppression of radiation-induced nf-kappab activity. Biomed Res Int *2015*: 363671, 2015. PMID: 26539482. DOI: 10.1155/2015/363671

9 Jeong YK, Oh JY, Yoo JK, Lim SH and Kim EH: The biofunctional effects of mesima as a radiosensitizer for hepatocellular carcinoma. Int J Mol Sci *21(3)*, 2020. PMID: 32013255. DOI: 10.3390/ijms21030871

10 Kuan LY, Chen WL, Chen JH, Hsu FT, Liu TT, Chen WT, Wang KL, Chen WC, Liu YC and Wang WS: Magnolol induces apoptosis and inhibits ERK-modulated metastatic potential in hepatocellular carcinoma cells. In Vivo *32(6)*: 1361-1368, 2018. PMID: 30348689. DOI: 10.21873/invivo.11387

11 Tsai JJ, Chen JH, Chen CH, Chung JG and Hsu FT: Apoptosis induction and erk/nf-kappab inactivation are associated with magnolol-inhibited tumor progression in hepatocellular carcinoma *in vivo*. Environ Toxicol *35(2)*: 167-175, 2020. PMID: 31714653. DOI: 10.1002/tox.22853

12 Brendan TM and ROBERT A. R. HURTA: Magnolol affects expression of IGF-1 and associated binding proteins in human prostate cancer cells *in vitro*. Anticancer Res *34(11)*: 6333-6338, 2014. PMID: 25368232.

13 Weng MC, Li MH, Chung JG, Liu YC, Wu JY, Hsu FT and Wang HE: Apoptosis induction and akt/nf-kappab inactivation are associated with regroafenib-inhibited tumor progression in non-small cell lung cancer *in vitro* and *in vivo*. Biomed Pharmacother *116*: 109032, 2019. PMID: 31163381. DOI: 10.1016/j.biopha.2019.109032

14 Tsai JJ, Hsu FT, Pan PJ, Chen CW and Kuo YC: Amentoflavone enhances the therapeutic efficacy of sorafenib by inhibiting anti-apoptotic potential and potentiating apoptosis in hepatocellular carcinoma *in vivo*. Anticancer Res *38(4)*: 2119-2125, 2018. PMID: 29599330. DOI: 10.21873/anticanres.12452

15 Hsu LC, Tu HF, Hsu FT, Yueh PF and Chiang IT: Beneficial effect of fluoxetine on anti-tumor progression on hepatocellular carcinoma and non-small cell lung cancer bearing animal model. Biomed Pharmacother *126*: 110054, 2020. PMID: 32145588. DOI: 10.1016/j.biopha.2020.110054

16 Chen JC, Chuang HY, Hsu FT, Chen YC, Chien YC and Hwang JJ: Sorafenib pretreatment enhances radiotherapy through targeting mek/erk/nf-kappab pathway in human hepatocellular carcinoma-bearing mouse model. Oncotarget *7(51)*: 85450-85463, 2016. PMID: 27863427. DOI: 10.18632/oncotarget.13398

17 Rahmanian N, Hosseinimehr SJ and Khalaj A: The paradox role of caspase cascade in ionizing radiation therapy. J Biomed Sci *23(1)*: 88, 2016. PMID: 27923354. DOI: 10.1186/s12929-016-0306-8

18 Magne N, Toillon RA, Bottero V, Didelot C, Houtte PV, Gerard JP and Peyron JF: Nf-kappab modulation and ionizing radiation: Mechanisms and future directions for cancer treatment. Cancer Lett *231(2)*: 158-168, 2006. PMID: 16399220. DOI: 10.1016/j.canlet.2005.01.022

19 Trivigno D, Essmann F, Huber SM and Rudner J: Deubiquitinase usp9x confers radioresistance through stabilization of mcl-1. Neoplasia *14(10)*: 893-904, 2012. PMID: 23097624. DOI: 10.1593/neo.12598

20 Nakazaki H: Preoperative and postoperative cytokines in patients with cancer. Cancer *70(3)*: 709-713, 1992. PMID: 1320454. DOI: 10.1002/1097-0142(19920801)70:3<709::aid-cncr2820700328>3.0.co;2-o

21 Tsai JJ, Pan PJ and Hsu FT: Regorafenib induces extrinsic and intrinsic apoptosis through inhibition of erk/nf-kappab activation in hepatocellular carcinoma cells. Oncol Rep *37(2)*: 1036-1044, 2017. PMID: 28000898. DOI: 10.3892/or.2016.5328

22 Liu YC, Wu RH and Wang WS: Regorafenib diminishes the expression and secretion of angiogenesis and metastasis associated proteins and inhibits cell invasion *via* nf-κb inactivation in sk-hep1 cells. Oncology letters *14(1)*: 461-467, 2017. PMID: 28693192. DOI: 10.3892/ol.2017.6142

23 Zhou K, Luo X, Wang Y, Cao D and Sun G: Microrna-30a suppresses tumor progression by blocking ras/raf/mek/erk signaling pathway in hepatocellular carcinoma. Biomed Pharmacother *93*: 1025-1032, 2017. PMID: 28732393. DOI: 10.1016/j.biopha.2017.07.029

24 Marampon F, Ciccarelli C and Zani BM: Biological rationale for targeting mek/erk pathways in anti-cancer therapy and to potentiate tumour responses to radiation. Int J Mol Sci *20(10)*, 2019. PMID: 31126017. DOI: 10.3390/ijms20102530