JBUON 2017; 22(3): 644-651
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Downregulation and subcellular distribution of HER2 involved in MDA-MB-453 breast cancer cell apoptosis induced by lapatinib/celastrol combination

Yan-yan Yan[1], Hong Bi[2], Wei Zhang[3], Qing Wen[3], Hong Liu[1], Jin-xia Li[1], Hui-zhi Zhang[1], Yan-xia Zhang[1], Jia-shan Li[1]

[1] Institute of Respiratory and Occupational Diseases; Collaborative Innovation Center for Cancer; Medical College, Shanxi Datong University, Datong, Shanxi, China.[2] Department of Pathology, Shanxi Provincial People's Hospital, Taiyuan, Shanxi, China.[3] Cancer Center of Datong, the Second People's Hospital of Datong, Shanxi, China.

Summary

Purpose: *To investigate the effect and related molecular mechanisms of lapatinib/celastrol combination or single-agent treatment in HER2/neu-overexpressing MDA-MB-453 breast cancer cells.*

Methods: *The effects of treatment with lapatinib, celastrol or their combination on cell growth were determined using MTT assay. Drug synergy was determined using the combination index (CI) methods derived from Chou-Talalay equations using CalcuSyn software. Apoptotic morphology was observed by fluorescence microscope with Hoechst 33258 staining. Changes of apoptotic and growth pathways-related proteins were analysed by Western blot. The expression of HER2 of cell surface was performed by flow cytometry. Subcellular distribution of HER2 was observed by immunofluorescence study.*

Results: *Combination celastrol and lapatinib produced strong synergy in growth inhibition and apoptosis in comparison to single-agent treatment in HER2/neu-overexpressing MDA-MB-453 cells. Interestingly, compared with celastrol treatment alone, lapatinib/celastrol combination induced more HER2 membrane protein downregulation and ectopic to cytoplasm and nucleus in MDA-MB-453 cells.*

Conclusion*: The combination of celastrol and lapatinib could be used as a novel combination regimen which provides a strong anticancer synergy in the treatment of HER2/neu-overexpressing cancer cells.*

Key words: *celastrol, HER2, lapatinib, MDA-MB-453 cells, synergy*

Introduction

It is well known that overexpression of HER2/neu is associated with a poor prognosis in many malignancies, including breast, ovarian, lung and prostate cancer, etc [1-4]. During the last decade, HER2/neu has been the focus for development of novel anticancer drugs in the form of small molecules (e.g. lapatinib) or monoclonal antibodies (e.g. herceptin), which have shown promising results [5,6]. However, their efficacy and long-term use in patients are quite limited due to resistance to these inhibitors or severe side effects. Therefore, novel therapeutic strategies in this field are still required.

Celastrol (Figure 1), purified from the plant Tripterygium wilfordii, also called the Thunder of God vine, has been used as a natural remedy in Chinese medicine for over 2,000 years. In the past decade, celastrol has become the focus of numerous preclinical studies that have shown its

Correspondence to: Yan-yan Yan, MD. Collaborative Innovation Center for Cancer, Medical College, Shanxi Datong University, Xing-yun Street, Datong 037009, People's Republic of China. Tel and Fax: +86 352 6011150, E-mail: zwsanyan@163.com
Received: 15/01/2017; Accepted: 30/01/2017

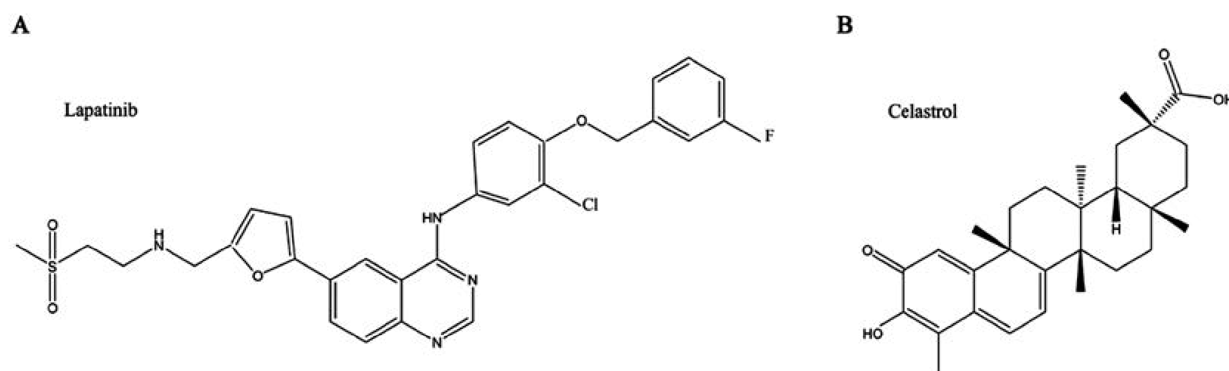


Figure 1. Chemical structure. **A:** lapatinib; **B:** celastrol.

potential for use in a wide range of conditions, from inflammatory diseases such as arthritis and Crohn's disease, to neurologic diseases such as Alzheimer's and amyotrophic lateral sclerosis [7,8]. More recently, both *in vitro* and *in vivo* studies have yielded results suggesting that celastrol may also be effective in the treatment of chemoresistant neoplasms including pancreatic cancer, glioma, and melanoma [9,10]. Preclinical studies in melanoma have shown that celastrol synergistically enhances temozolomide cytotoxicity in melanoma cells [9].

Our previous data have shown that celastrol enhanced the anticancer effect of lapatinib in HepG2 human hepatocellular carcinoma cells *in vitro* [11]. In this study, we futher investigated the effect and related molecular mechanisms of lapatinib/celastrol combination in HER2/neu-overexpressing MDA-MB-453 cells.

Methods

Chemicals and Reagents

Lapatinib was purchased from Glaxo Smith Klein Co (Philadelphia, PA, USA). Celastrol was purchased from PayPay Technologies (Guangdong, China). Anti-mouse IgG-fluorescein isothiocyanate (FITC) (for immunofluorescence assay), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and Hoechst 33258 were purchased from Sigma (St. Louis, MO, USA). Antibodies against caspase-9, caspase-3 and p-HER2 were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against HER2 for flow cytometry and western blot were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against p-Akt, p-ERK1/2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), anti-mouse IgG-horseradish peroxidase, and anti-rabbit IgG-horseradish peroxidase were purchased from KangChen Biotechnology (Shanghai, China). All tissue culture supplies were purchased from Life Technologies (Carlsbad, CA, USA). Other routine laboratory reagents of analytical or high-performance liquid chromatography grade were purchased from Whiga Biotechnology (Guangzhou, China).

Cell lines and cell culture

Human breast cancer cell lines MDA-MB-453 obtained from cell bank of Chinese Academy of Medical Sciences (Beijing, China) were grown in DMEM medium containing 100 U/mL penicillin, 100 mg/mL streptomycin, and 10% fetal bovine serum (FBS). Cells were cultured in a humidified atmosphere incubator of 5% CO_2 and 95% air at 37℃.

Cytotoxicity assays and analysis of drug synergy

The effects of treatment with lapatinib and/or celastrol on cell growth were determined using MTT assay [12]. After 68-h treatment, 20 μL MTT (5 mg/mL stock solution of saline) were added to each well for 4 hrs. Subsequently, the supernatant was removed, and MTT crystals were solubilized with 100μL anhydrous DMSO in each well. Thereafter, cell viability was measured by model 550 microplate reader (Bio-Rad, Hercules, CA, USA) at 540 nm, with 655 nm as reference filter. The 50% inhibitory concentration (IC_{50}) was determined as the anticancer drug concentration causing 50% reduction in cell viability and calculated from the cytotoxicity curves (Bliss' software). Percent cell survival was calculated using the following formula: survival (%) = [(mean experimental absorbance) / (mean control absorbance)]×100%. Drug synergy was determined using combination index (CI) methods derived from Chou-Talalay equations [13] using CalcuSyn software (Biosoft, Cambridge, UK). A CI value of 1 indicates an additive effect between two agents, whereas a CI value of <1 indicates synergy.

Assessment of apoptosis morphology by Hoechst 33258 staining

MDA-MB-453 cells were treated with the indicated concentrations of lapatinib and/or celastrol for 48 hrs. Both floating and trypsinized adherent cells were collected, washed once with ice-cold PBS, fixed with 1 mL of 4% paraformaldehyde for 20 min, and washed once with ice-cold PBS. Then, the cells were incubated in 1 mL PBS

containing 10 μmol/L Hoechst 33258 at 37℃ for 30 min, washed twice, and observed using fluorescence microscopy with standard excitation filters (Leica, Germany) in random microscopic fields at × 400 magnification.

Whole-cell lysates and Western blot analysis

After MDA-MB-453 cells were exposed to the indicated concentrations of lapatinib and/or celastrol for 48 hrs, whole cells were harvested and washed twice with ice-cold PBS, the pellet was vortexed and 1× lysis buffer [50 mmol/L Tris- HCl (PH 6.8), 10% glycerol, 2% SDS, 0.25% bromophenol blue, and 0.1 mol/L DTT] was added for 100 μL/ 5×10^6 cells. After heated at 95℃ for 20 min, the lysates were centrifuged at 12,000 rpm for 10 min and the supernatant was collected. The protein concentration was determined by nucleic acid-protein analyzer (Beckman, CA, USA). Equal amount of lysate protein was separated on 8% to 12% SDS-PAGE and transferred onto polyvinylidene difluoride membrane (Pall). The nonspecific binding sites were blocked with Tris-Buffered Saline and Tween 20 (TBST) buffer [150 mmol/L NaCl, 20 mmol/L Tris-HCl (pH 7.4), and 0.4% (v/v) Tween 20] containing 5% nonfat dry milk for 2 hrs. The membranes were incubated overnight at 4℃ with specific primary antibodies. Then, the membranes were washed three times with TBST buffer and incubated at room temperature for 1 hr with horseradish peroxidase-conjugated secondary antibody. After three washes with TBST buffer, the immunoblots were visualized by the enhanced Phototope-Horseradish Peroxidase Detection Kit purchased from Cell Signaling Technology (Danvers, MA, USA) and exposed to Kodak medical X-ray processor (Rochester, NY, USA) [14].

Expression of HER2 protein analysed by flow cytometry

Determination of the expression of HER2 of cell surface was performed according to manufacture's instruction. MDA-MB-453 cells (6×10^5) were seeded in 25 cm^2 flasks and allowed to attach. After treated with the indicated concentrations of lapatinib and/or celastrol for 48 hrs, both floating and attached cells were collected and washed with ice-cold PBS twice. Single-cell suspensions were prepared by the addition of 0.5 mmol/L EDTA followed by three washes with an isotonic PBS buffer [supplemented with 0.5% bovine serum albumin (BSA)]. Then, MDA-MB-453 cells (100μL) were incubated at 4℃ for 45 min with 20μL of R-Phytoerythrin (R-PE)-conjugated anti-human HER2 reagent. Following this incubation, cells were washed twice with PBS buffer (supplemented with 0.5% BSA) and the supernatant was discarded. Finally, the cells were resuspended in 400 μL PBS buffer for flow cytometric analysis. Isotype control samples were treated in an identical manner with PE-labeled mouse IgG2bκ antibody [15].

Immunofluorescence assay

After MDA-MB-453 cells grown on coverslips were treated with the indicated concentrations of lapatinib and/or celastrol for 48 hrs, cells were fixed with 4% polyoxymethylene for 20 min, and Triton X-100 was added for 10 min at room temperature. Then, the cells were rinsed with PBS for three times, and the nonspecific binding sites were blocked in PBS with 1% BSA for 1 hr. The cells were incubated overnight at 4℃ with HER2 antibody followed by FITC-conjugated secondary antibody at room temperature for 1 hr. Subcellular distribution of HER2 protein was observed under fluorescence microscopy with standard excitation filters (Leica Dmirb) in random microscopic field at × 400 magnification [14].

Statistics

For each protocol, three independent experiments were performed. The results were expressed as mean±standard error of the mean (SEM). Statistical calculations were performed by using SPSS16.0 software. Differences in measured variables between experimental and control groups were assessed by the Student's t-test and $p<0.01$ was indicative of very significant statistical difference.

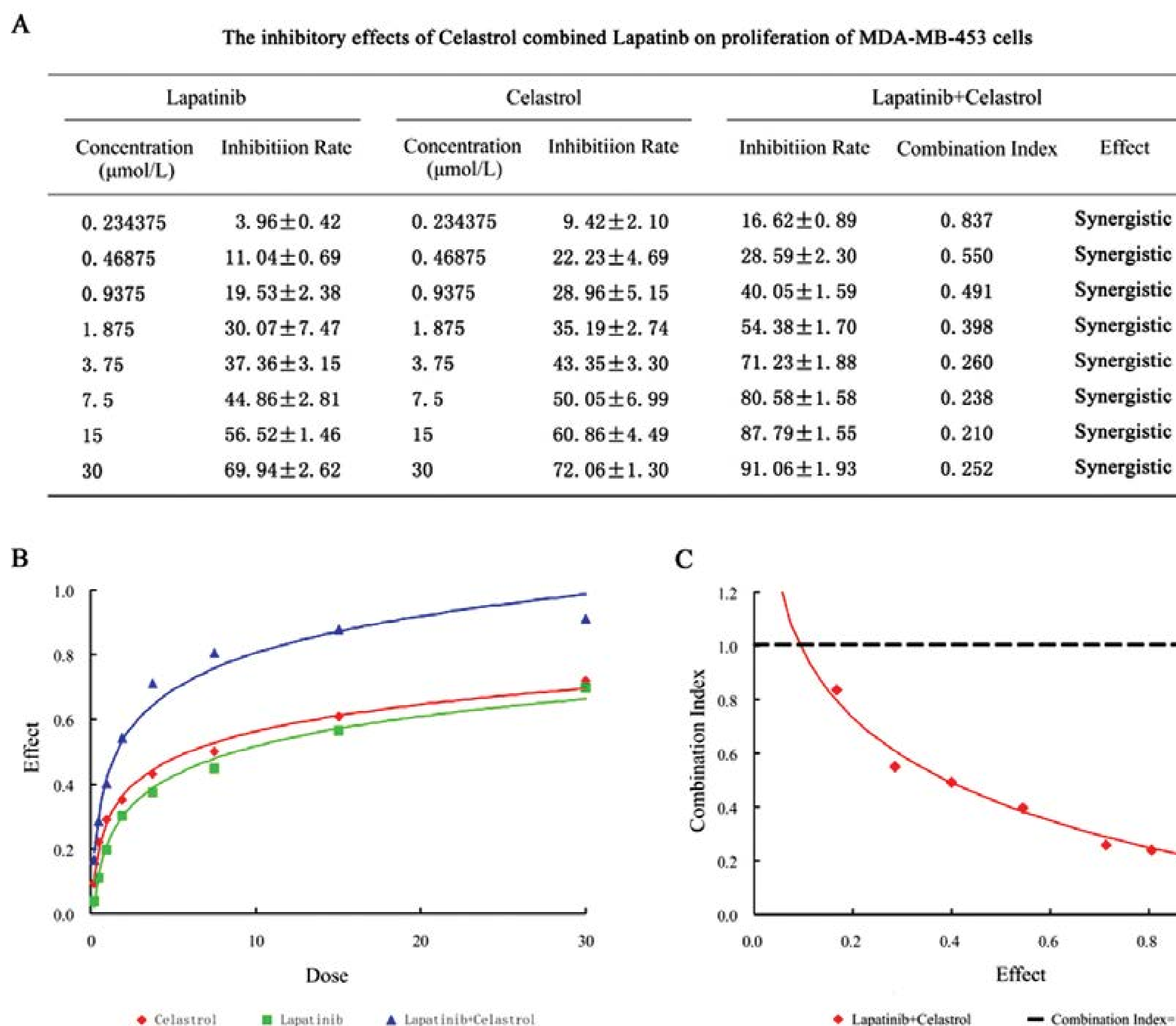
Results

Lapatinib/celastrol synergistically inhibit cell proliferation in MDA-MB-453 cells

The growth inhibitory effects on MDA-MB-453 cells of lapatinib and/or celastrol were determined using MTT assay. Our results showed that the combination treatment with lapatinib/celastrol yielded significantly greater growth inhibition than lapatinib or celastrol treatment alone (Figure 2A and B). As shown in Figure 2A and C, CI values were <1 at all doses of lapatinib tested (range, 0.234375-30μmol/L), indicating that celastrol (range, 0.234375-30μmol/L) was synergistic with lapatinib across a broad range of concentrations.

Lapatinib/celastrol combination synergistically induced MDA-MB-453 cells apoptosis

To assess the ability of celastrol to sensitize MDA-MB-453 cells to lapatinib, we observed the morphologic characteristics of apoptosis. Control cells showed even distribution of the stain and round homogeneous nuclei features. Apoptotic cells displayed typical changes including reduction of cellular volume, staining bright and condensed or fragmented nucleus. More apoptotic bodies were observed in the lapatinib/celastrol combination compared with lapatinib or celastrol treatment alone (Figure 3). Western blot further



A

The inhibitory effects of Celastrol combined Lapatinb on proliferation of MDA-MB-453 cells

Lapatinib		Celastrol		Lapatinib+Celastrol		
Concentration (μmol/L)	Inhibitiion Rate	Concentration (μmol/L)	Inhibitiion Rate	Inhibitiion Rate	Combination Index	Effect
0. 234375	3. 96±0. 42	0. 234375	9. 42±2. 10	16. 62±0. 89	0. 837	Synergistic
0. 46875	11. 04±0. 69	0. 46875	22. 23±4. 69	28. 59±2. 30	0. 550	Synergistic
0. 9375	19. 53±2. 38	0. 9375	28. 96±5. 15	40. 05±1. 59	0. 491	Synergistic
1. 875	30. 07±7. 47	1. 875	35. 19±2. 74	54. 38±1. 70	0. 398	Synergistic
3. 75	37. 36±3. 15	3. 75	43. 35±3. 30	71. 23±1. 88	0. 260	Synergistic
7. 5	44. 86±2. 81	7. 5	50. 05±6. 99	80. 58±1. 58	0. 238	Synergistic
15	56. 52±1. 46	15	60. 86±4. 49	87. 79±1. 55	0. 210	Synergistic
30	69. 94±2. 62	30	72. 06±1. 30	91. 06±1. 93	0. 252	Synergistic

Figure 2. Lapatinib in combination with celastrol synergistically induced cytotoxicity in MDA-MB-453 cells. MDA-MB-453 cells were treated with lapatinib as a single drug or in combination with celastrol as described in the methods section. Lapatinib plus celastrol combination included variable celastrol (0.234375 to 30 μmol/L) and lapatinib (0.234375 to 30 μmol/L) concentrations. Controls included untreated and DMSO-treated cells. Growth inhibition of cells were then evaluated by MTT assay. Shown here is the comparison of cytotoxicity of single drug and the lapatinib/celastrol combination treatment. **A:** Chou-Talalay analyses for celastrol plus lapatinib combination. Combination index (CI)<1 indicates synergy; **B:** Dose-effect plot. There are obvious differences between groups (p<0.01). **C:** CI-effect plot. The dotted line represents CI=1. Correlation analysis indicates significantly negative correlation between the inhibition effect of lapatinib/celastrol combination treatment and CI (p<0.01).

revealed that when the cells were treated with the combination of celastrol (5μmol/L) and lapatinib (5μmol/L) a strong synergy in growth inhibition and apoptosis *in vitro* in comparison to single treatments was shown, indicating more activation of apoptotic related proteins (caspase-9 and caspase-3) and downregulation of growth pathway related proteins (HER2, P-HER2, p-Akt, p-ERK1/2) (Figure 4).

Lapatinib enhanced the ability of celastrol to decrease HER2 membrane protein expression in MDA-MB-453 cells

To further investigate the mechanism of lapatinib/celastrol combination that synergistically induced MDA-MB-453 cell apoptosis, we also determined the expression of HER2 of cell surface. Flow cytometry revealed that treating the cells with the combination of celastrol (5μmol/L) and lapatinib (5μmol/L) produced strong synergy in decreasing the expression of HER2 of the cell surface compared with celastrol or lapatinib alone (Figure 5). Isotype control samples were treated in an identical manner with PE-labeled mouse IgG2bκ antibody. The expression levels of HER2 of the cell surface were 94.77±1.15% for the control, 87.13±1.31% for lapatinib alone, 30.23±0.93% for celastrol alone, and 20.57±1.72% for lapatinib plus celastrol combination, respectively.

More changes of subcellular distribution of HER2 induced by lapatinib/celastrol combination in MDA-MB-453 cells

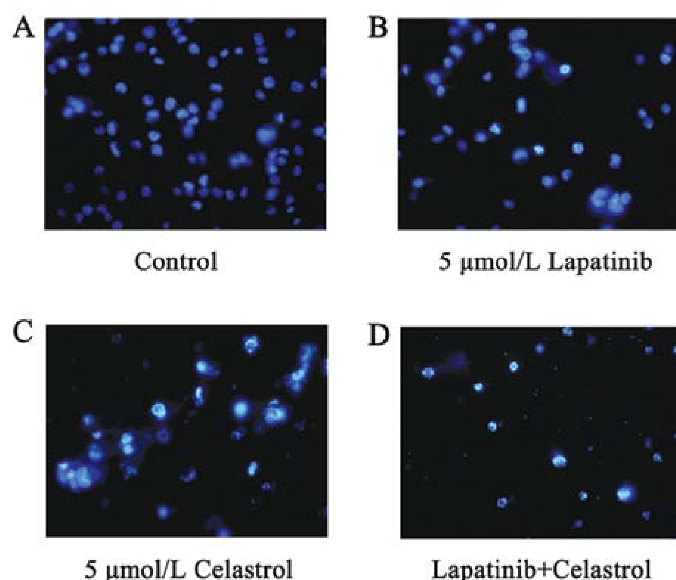


Figure 3. Combination of celastrol and lapatinib more markedly induced apoptosis morphologic changes than lapatinib or celastrol alone in MDA-MB-453 cells. The apoptotic bodies of MDA-MB-453 cells were detected by Hoechst 33258 staining and observed under fluorescence microscope in random microscopic fields at ×400 magnification. **A:** Control. **B:** 5μmol/L lapatinib. **C:** 5μmol/L celastrol. **D:** 5μmol/L lapatinib + 5μmol/L celastrol.

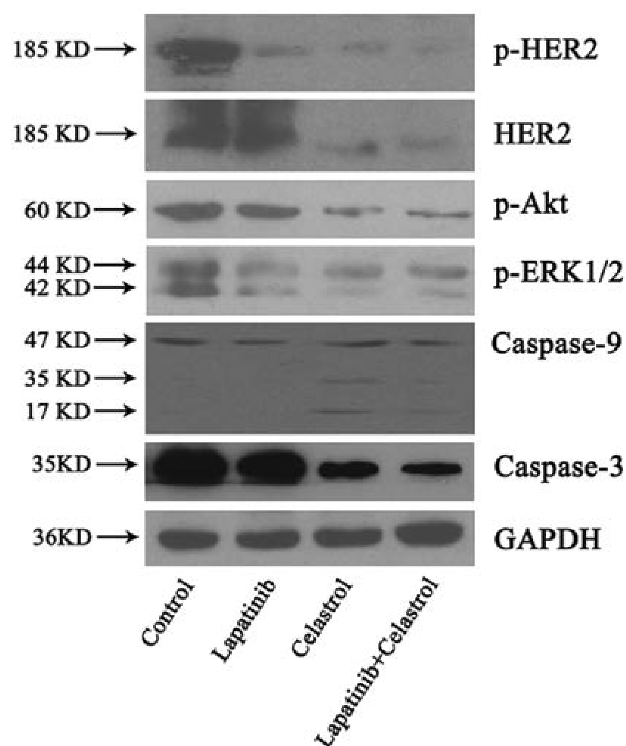


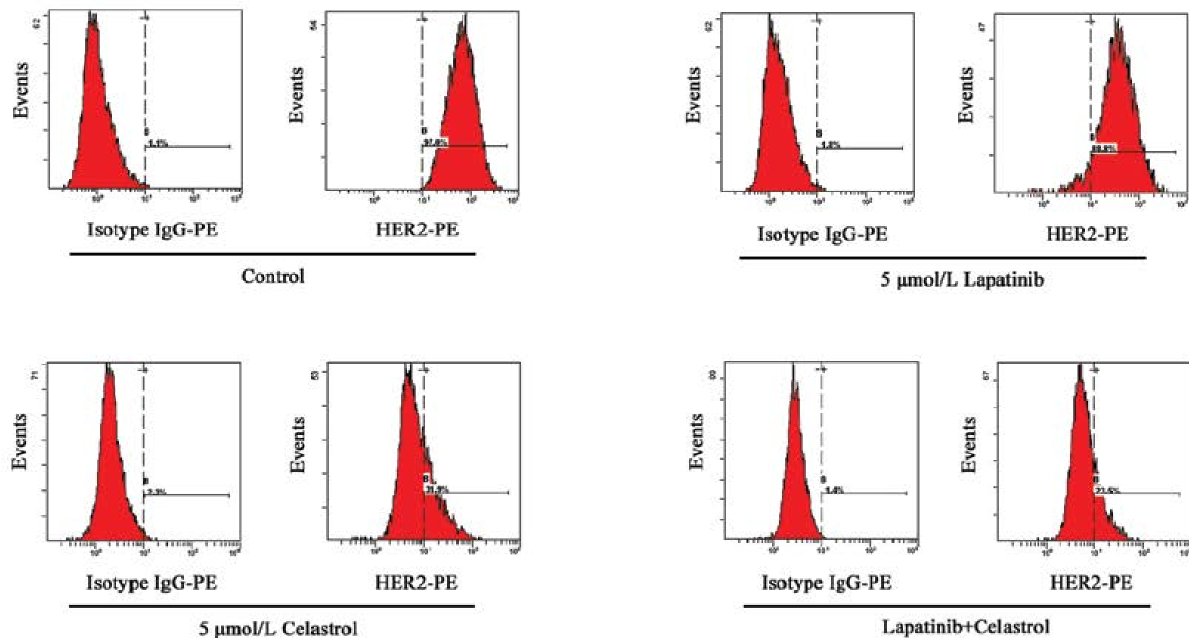
Figure 4. Celastrol enhanced lapatinib-induced apoptosis and reduced tumor growth in MDA-MB-453 cells. Cells were treated with indicated concentrations of lapatinib and/or celastrol for 48 hrs. Changes of apoptotic and growth pathways-related proteins were analysed by Western blot. GAPDH was used as a loading control.

To further investigate the mechanism of the downregulation of HER2 protein, immunofluorescence study with anti-HER2/neu antibody was also performed. As shown in Figure 6, the control cells had strong immunofluorescence at the plasma membrane. After celastrol treatment, the immunofluorescence at the plasma membrane was attenuated and replaced with diffuse cytoplasmic and nuclear punctate staining. More changes of subcellular distribution of HER2 were observed by lapatinib/celastrol combination than celastrol treatment alone in MDA-MB-453 cells.

Discussion

Molecular targeting therapy for cancer treatment is a fast-growing research field in oncology [6]. Lapatinib is an inhibitor of the intracellular tyrosine kinase domains of both the EGFR and HER2 receptors. Mutations or dysregulation in these receptors has been shown to play a role in the development of certain cancers. Lapatinib was approved for use in combination with capecitabine for the treatment of patients with advanced or metastatic breast cancer whose tumors overexpressed HER2 and who had received prior therapy with anthracycline, taxane, and trastuzumab. As the new tyrosine kinase inhibitors (TKIs) are being introduced into the clinic, a significant effort will be directed toward increasing the anticancer activity of conventional chemotherapeutic agents or restoring chemosensitivity of resistant cancer cells to conventional chemotherapeutic agents [14]. However, due to the heterogeneous and dynamic nature of tumors, the effectiveness of these agents is often hindered by poor response rates and acquired drug resistance. Several strategies have been proposed to overcome the low response rate and acquired resistance to TKIs. One particularly promising approach is the modulation of TKI pathways by inhibiting the expression of HER2. To further explore the versatility of this idea, we proposed a novel strategy with celastrol to improve lapatinib therapy in HER2/neu-overexpressing MDA-MB-453 cells.

Celastrol is a natural product used in traditional Chinese medicine that has demonstrated ability to inhibit cancer progression and downregulate NF-kappa B activity in prostate cancer and leukemia cells [16-18]. Our previous data has shown that celastrol enhanced the anticancer effect of lapatinib in HepG2 human hepatocellular carcinoma cells *in vitro* [10]. In this study, we further investigated the effect and related molecular mechanisms of lapatinib/celastrol combination in HER2/neu-overexpressing MDA-MB-453 cells.



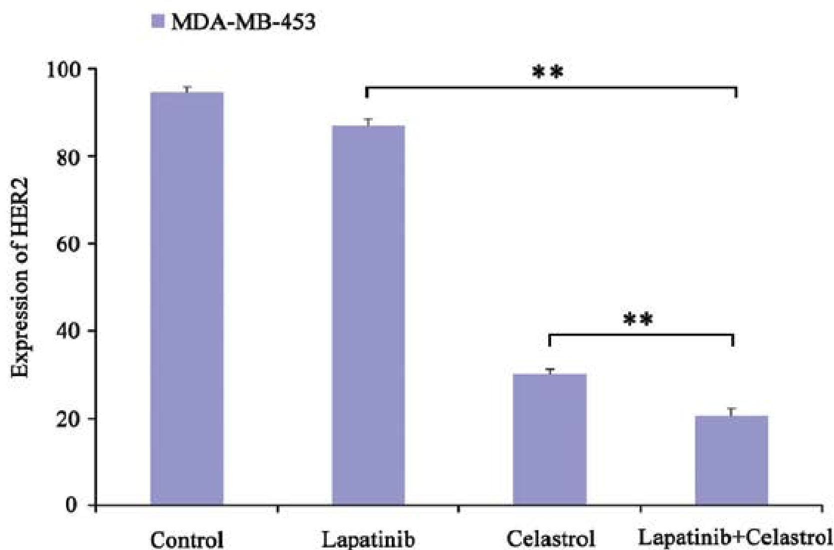


Figure 5. Combination of celastrol and lapatinib further induced decrease of HER2 membrane protein in MDA-MB-453 cells. **A:** Determination of the expression of HER2 of cell surface by flow cytometry; isotype control samples were treated in an identical manner with PE-labeled mouse IgG2bκ antibody. **B:** Statistical analysis of the expression of HER2. Mean±SD of three assays. ** $p<0.01$ compared with lapatinib/celastrol combination treatment, n=3.

Our results showed for the first time that the combination of lapatinib and celastrol synergistically inhibited the proliferation and induced apoptosis in MDA-MB-453 cells *in vitro*. Treatment with celastrol plus lapatinib was synergistic in inhibiting MDA-MB-453 cell growth across a broad range of concentrations (Figure 2). More apoptotic bodies were observed in the lapatinib/celastrol combination compared with lapatinib or celastrol treatment alone (Figure 3). Moreover, combination treatment with lapatinib/celastrol resulted in lower levels of HER2 of the cell surface compared

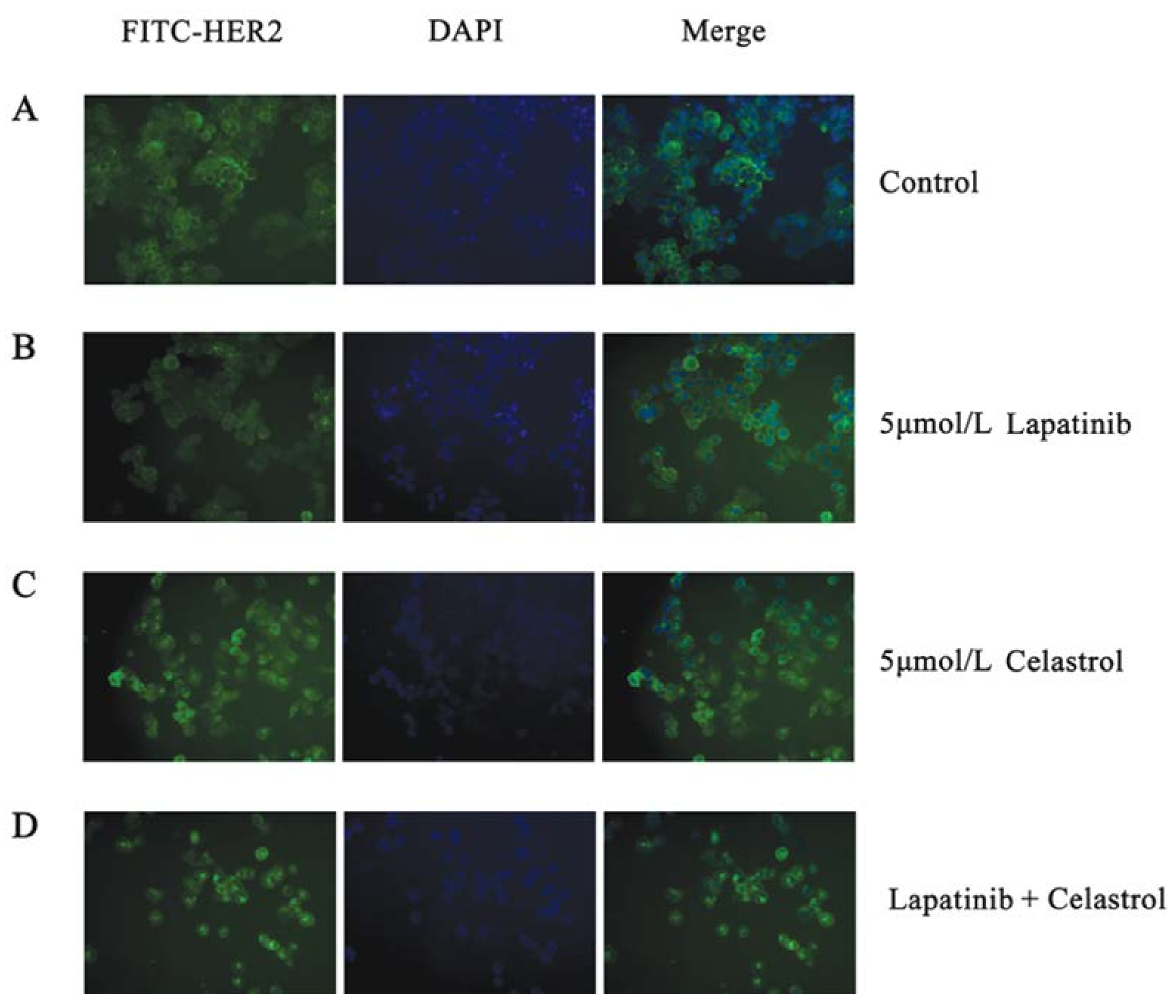


Figure 6. Combination of celastrol and lapatinib significantly altered the subcellular distribution of HER2. After MDA-MB-453 cells grown on coverslips were treated with the indicated concentrations of lapatinib and/or celastrol for 48 hrs, immunofluorescence assay was performed according to the methods section. **A:** Control. **B:** 5μmol/L lapatinib. **C:** 5μmol/L celastrol. **D:** 5μmol/L lapatinib + 5μmol/L celastrol.

with either agent alone (Figure 5). Interestingly, more changes of subcellular distribution of HER2 were observed by lapatinib/celastrol combination than celastrol treatment alone in MDA-MB-453 cells (Figure 6). Western blotting further revealed that the combination of celastrol and lapatinib produced strong synergy in cell growth inhibition and apoptosis-inducing abilities *in vitro* in comparison to single treatments, showing more activation of apoptotic related proteins (caspase-9 and caspase-3) and downregulation of growth pathway related proteins (HER2, P-HER2, p-Akt, p-ERK1/2) (Figure 4), implicating activation of mitochondrial-dependent cell apoptosis pathway and inhibition of the PI3K/Akt and MAPK proliferation pathway maybe involved in the observed synergistic effects. These results also indicated that lapatinib's antitumor effect (inhibiting HER2 activity) could be amplified by celastrol (downregulating the expression of HER2) in MDA-MB-453 cells.

In summary, our results indicated that celastrol could enhance the cytotoxicity and apoptosis-inducing effect of lapatinib in HER2/neu-overexpressing MDA-MB-453 cells. Mechanistic studies consistently showed that celastrol not only directly inhibited the expression of HER2 and changed its redistribution, but also had the potential to achieve further synergy in tumor suppression when combined with lapatinib. Celastrol may also be useful in combination therapies by enhancing the efficacy of targeting agents. This work also showed a practical approach to enhance HER2/neu-overexpressing cancer therapy.

Acknowledgements

This work was supported by the National Sci-

ence Foundation for Young Scientists of Shanxi Province (2014021037-6), Science and Technology Research Project of Shanxi Province (20140313011-2), the Research Foundation of Collaborative Innovation Center for Cancer and the Priming Scientific Research Foundation for Ph.D. in Shanxi Datong University (No. 2010-B-11)

Conflict of interests

The authors declare no confict of interests.

References

1. Oshima RG, Lesperance J, Munoz V et al. Angiogenic acceleration of Neu induced mammary tumor progression and metastasis. Cancer Res 2004;64:169-179.
2. Slamon DJ, Godolphin W, Jones LA et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244:707-712.
3. Nakamura H, Saji H, Ogata A et al. Correlation between encoded protein overexpression and copy number of the HER2 gene with survival in non-small cell lung cancer. Int J Cancer 2003;103: 61-66.
4. Menard S, Pupa SM, Campiglio M, Tagliabue E. Biologic and therapeutic role of HER2 in cancer. Oncogene 2003;22:6570-6578.
5. Rowinsky EK. The erbB family: targets for therapeutic development against cancer and therapeutic strategies using monoclonal antibodies and tyrosine kinase inhibitors. Annu Rev Med 2004;55:433-457.
6. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. N Engl J Med 2005;353:172-187.
7. Kiaei M, Kipiani K, Petri S, Chen J, Calingasan NY, Beal MF. Celastrol blocks neuronal cell death and extends life in transgenic mouse model of amyotrophic lateral sclerosis. Neurodegener Dis 2005;2:246-254.
8. Li H, Zhang YY, Tan HW, Jia YF, Li D. Therapeutic effect of tripterine on adjuvant arthritis in rats. J Ethnopharmacol 2008;118:479-484.
9. Abbas S, Bhoumik A, Dahl R et al. Preclinical studies of celastrol and acetyl isogambogic acid in melanoma. Clin Cancer Res 2007;13:6769-6778.
10. Huang Y, Zhou Y, Fan Y, Zhou D. Celastrol inhibits the growth of human glioma xenografts in nude mice through suppressing VHER2 expression. Cancer Lett 2008;264:101-106.
11. Yan YY, Guo Y, Zhang W et al. Celastrol enhanced the anticancer effect of lapatinib in human hepatocellular carcinoma cells in vitro. JBUON 2014;19:412-418.
12. Yan Y, Su X, Liang Y et al. Emodin azide methyl anthraquinone derivative triggers mitochondrial-dependent cell apoptosis involving in caspase-8-mediated Bid cleavage. Mol Cancer Ther 2008;7:1688-1697.
13. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 1984;22:27-55.
14. Yan YY, Zheng LS, Zhang X et al. Blockade of Her2/neu Binding to Hsp90 by Emodin Azide Methyl Anthraquinone Derivative Induces Proteasomal Degradation of Her2/neu. Mol Pharm 2011;8:1687-1697.
15. Dai CL, Tiwari AK, Wu CP et al. Lapatinib (Tykerb, GW572016) reverses multidrug resistance in cancer cells by inhibiting the activity of ATP-binding cassette subfamily B member 1 and G member 2. Cancer Res 2008;68:7905-7914.
16. Setty AR, Sigal LH. Herbal medications commonly used in the practice of rheumatology: mechanisms of action, efficacy, and side effects. Semin Arthritis Rheum 2005;34:773-784.
17. Lee JH, Koo TH, Yoon H et al. Inhibition of NF-kappa B activation through targeting I kappa B kinase by celastrol, a quinone methide triterpenoid. Biochem Pharmacol 2006;72:1311-1321.
18. Yang H, Chen D, Cui QC, Yuan X, Dou QP. Celastrol, a triterpene extracted from the Chinese “Thunder of God Vine,” is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice. Cancer Res 2006;66:4758-4765.