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Y-27632 Increases Sensitivity of PANC-1 Cells to Epigallocatechin Gallate (EGCG) in Regulating Cell Proliferation and Migration

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: The study aimed to investigate the inhibitory effect of (1R,4r)-4-((R)-1-aminoethyl)-N-(pyridin-4-yl) cyclohexanecarboxamide (Y-27632) and (–)-epigallocatechin-3-gallate (EGCG) on the proliferation and migration of PANC-1 cells. EGCG, found in green tea, has been previously shown to be one of the most abundant and powerful catechins in cancer prevention and treatment. Y-27632, a selective inhibitor of rho-associated protein kinase 1, is widely used in treating cardiovascular disease, inflammation, and cancer.

Material/Methods: PANC-1 cells, maintained in Dulbecco's Modified Eagle's Medium, were treated with dimethyl sulfoxide (control) as well as different concentrations (20, 40, 60, and 80 μg/mL) of EGCG for 48 h. In addition, PANC-1 cells were treated separately with 60 μg/mL EGCG, 20 μM Y-27632, and EGCG combined with Y-27632 (60 μg/mL EGCG + 20 μM Y-27632) for 48 h. The effect of EGCG and Y-27632 on the proliferation and migration of PANC-1 cells was evaluated using Cell Counting Kit-8 and transwell migration assays. The expression of peroxisome proliferator–activated receptor alpha (PPARα) and Caspase-3 mRNA was determined by Quantitative real-time polymerase chain reaction (RT-qPCR).

Results: EGCG (20–80 μg/mL) inhibited cell viability in a dose-dependent manner. Y-27632 enhanced the sensitivity of PANC-1 cells to EGCG (by increasing the expression of PPARα and Caspase-3 mRNA) and suppressed cell proliferation. PANC-1 cell migration was inhibited by treatment with a combination of EGCG and Y-27632.

Conclusions: Y-27632 increases the sensitivity of PANC-1 cells to EGCG in regulating cell proliferation and migration, which is likely to be related to the expression of PPARα mRNA and Caspase-3 mRNA.

MeSH Keywords: Cell Migration Assays • Cell Proliferation • Pancreatic Neoplasms

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Background

Pancreatic cancer, currently one of the most lethal human malignancies [1], is largely refractory to conventional therapies. Peroxisome proliferator-activated receptor alpha (PPARα), a member of the PPAR family [2], regulating tumorigenesis [3], is a ligand-activated transcription factor [4]. Caspase-3 is encoded by the CASP3 gene [5], as a potential therapeutic target for cancer patients [6] and plays key roles in the growth stimulation.

(–)-Epigallocatechin-3-gallate (EGCG) ($C_{22}H_{18}O_{11}$; Figure 1A), found in green tea [7], which is widely consumed in China [8], is one of the most abundant and powerful catechins [9] in cancer prevention and treatment. (1R,4r)-4-((R)-1-aminoethyl)-N-(pyridin-4-yl) cyclohexane carboxamide (Y-27632) ($C_{14}H_{21}N_3O$; Figure 1B), a selective inhibitor of rho-associated protein kinase 1 (ROCK1) [10], is widely used in treating cardiovascular disease [11], inflammation [12], and cancer [13]. Although Y-27632 [14–16] and EGCG [17–19] inhibit the growth of many cancer cells, whether the efficacy of Y-27632 increases the sensitivity of PANC-1 cells to EGCG is not yet clear. The present study hypothesized that the combination of Y-27632 and EGCG would reveal additive inhibitory effects *in vitro*.

In the present study, the capacity of Y-27632 to sensitize PANC-1 cells to the effects of EGCG in regulating cell proliferation and migration was investigated. Furthermore, the expression of PPARα *mRNA* and caspase-3 *mRNA* in EGCG and Y-27632 alone, and in EGCG combined with Y-27632 on PANC-1 cells, was examined.

Material and Methods

Cell culture

PANC-1 cells (SIBCB, Shanghai, China) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco BRL, MD, USA) (15) supplemented with 10% fetal bovine serum (Gibco BRL, MD, USA) and penicillin (100 U/mL)–streptomycin (100 mg/mL) (Gibco BRL, MD, USA) in a humidified atmosphere containing 5% CO_2 and 95% air at 37°C.

Cell proliferation assay

PANC-1 cells (1×10^6/well) were seeded into 96-well plates (Corning, NY, USA). These cells were then treated with dimethyl sulfoxide (DMSO) (control) as well as different concentrations (20, 40, 60, and 80 μg/mL) of EGCG (NICPBP, Beijing, China) for 48 h. In addition, PANC-1 cells were treated separately with DMSO (control), 60 μg/mL EGCG, 20 μM Y-27632, and EGCG combined with Y-27632 (60 μg/mL EGCG + 20 μM Y-27632) for 48 h. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) [16] as described in a previous study. The absorbance (*A*) of each hole in the 96-well plate was determined at 475 nm using a microplate reader, using the formula:

$$\text{Cell inhibition rate (\%)} = 1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\%$$

Hoechst 33258 staining

PANC-1 cells were treated separately with DMSO (control), 60 μg/mL EGCG, 20 μM Y-27632, and 60 μg/mL EGCG + 20 μM Y-27632 for 48 h. After washing with phosphate-buffered saline (Gibco BRL), the fixed PANC-1 cells were stained with 10 -μg/mL Hoechst 33258 (Beyotime Institute of Biotechnology, Jiangsu, China) [17] for 10 min at room temperature.

Transwell migration assay

PANC-1 cells (1×10^6/well) were placed in the upper chamber of a transwell filter. Drugs (DMSO, 60 μg/mL EGCG, 20 μM Y-27632, and 60 μg/mL EGCG + 20 μM Y-27632) were added separately into the upper chamber and PANC-1 cells were incubated for 10 h. After fixation and 0.1% crystal violet staining, PANC-1 cells were counted and the cell migration inhibition rate [18] of each group was calculated.

Quantitative real-time reverse transcription-polymerase chain reaction

PANC-1 cells were treated separately with DMSO (control), 60 μg/mL EGCG, 20 μM Y-27632, and 60 μg/mL EGCG + 20 μM Y-27632 for 48 h. Quantitative real-time polymerase chain reaction (RT-qPCR) [19] was used to observe the expression of PPARα mRNA and caspase-3 mRNA of these groups. The primer pairs [glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), PPARα, and caspase-3] were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The primer pairs included the following: forward: 5'-AGAAGGCTGGGGCTCATTTG-3' and reverse: 5'-AGGGGCCATCCACAGTCTTC-3' for *GAPDH* (258 bp); forward: 5'-TTCGCAATCCATCGGCGAG-3' and reverse: 5'-CCACAGGATAAGTCACCGAGG-3' for PPARα (146 bp). Forward: 5'- CATGGAAGCGAATCAATGGACT-3' and reverse: 5'-CTGTACCAGACCGAGATGTCA-3' for caspase-3 (139 bp). *GAPDH* was used as an internal control to evaluate the relative expression of PPARα. RT-qPCR reagents were purchased from TIANGEN Biotech (Beijing) Co., Ltd. (Beijing, China). Relative mRNA was calculated using the formula: $2^{-\Delta\Delta Ct}$ [20,21].

Statistical analysis

Data are shown as mean ± standard deviation. Statistical comparisons were performed using SPSS version 18.0 software (22). $P<0.05$ was considered to be significant [23,24].

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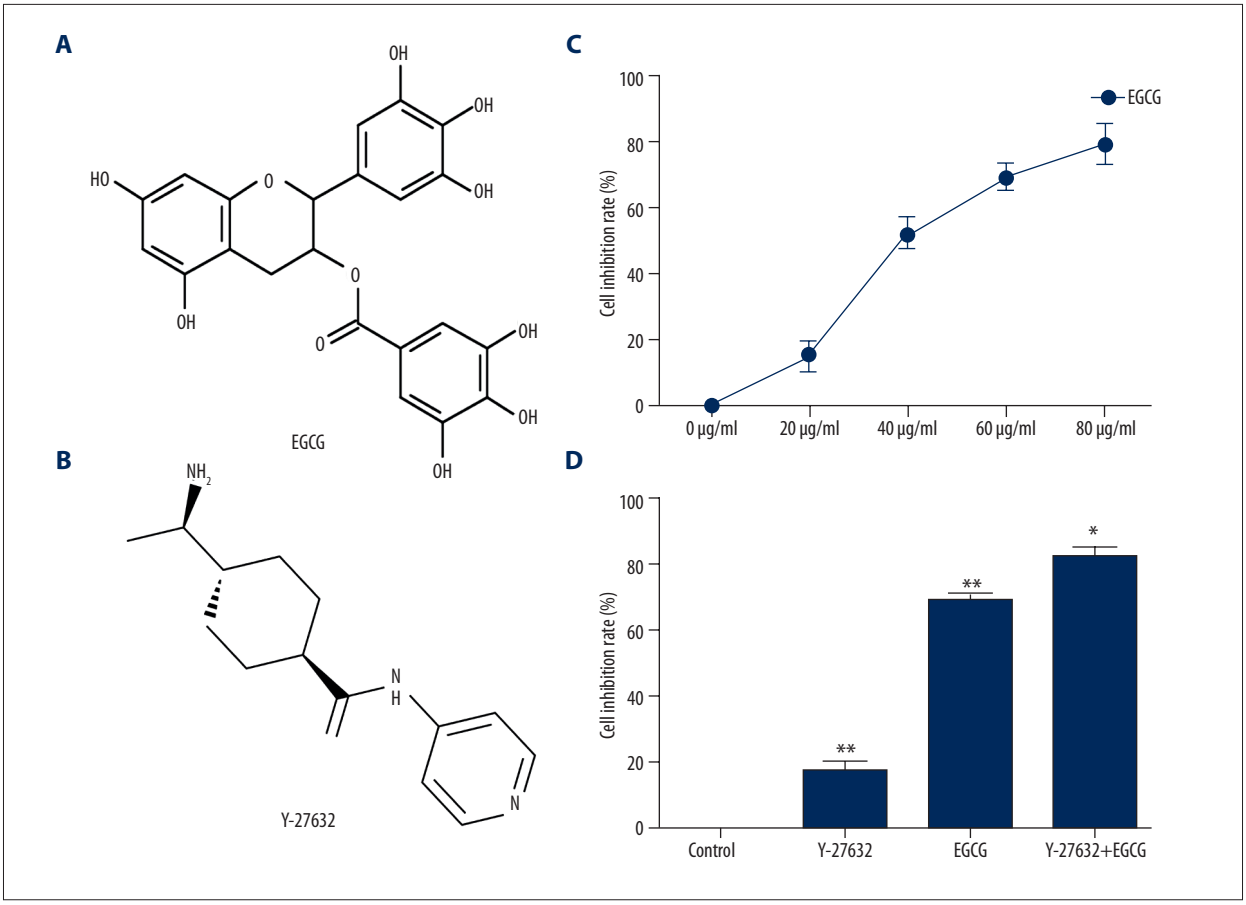

Figure 1. Effect of the combination of (–)-epigallocatechin-3-gallate (EGCG) and (1R,4r)-4-((R)-1-aminoethyl)-N-(pyridin-4-yl) cyclohexane carboxamide (Y-27632) on PANC-1 cell proliferation. (**A, B**) Chemical structure of EGCG ($C_{22}H_{18}O_{11}$) and Y-27632 ($C_{14}H_{21}N_3O$). (**C**) Different concentrations of EGCG (20, 40, 60, and 80 μg/mL) inhibited cell viability in a dose-dependent manner. The effect of EGCG and Y-27632 on the PANC-1 cell proliferation was evaluated by CCK-8 assay. (**D**) PANC-1 cells were treated separately with DMSO (control), 60 μg/mL EGCG, 20 μM Y-27632, and 60 μg/mL EGCG + 20 μM Y-27632 for 48 h. Data represent mean ± standard error of mean, n = 3. Compared with control, ** P<0.01. Compared with EGCG, * P<0.05.

Results

Y-27632 augments the antiproliferative effect of EGCG in PANC-1 cells

The effect of EGCG and Y-27632 on the PANC-1 cell proliferation was evaluated using the CCK-8 assay. Different concentrations of EGCG (20, 40, 60, and 80 μg/mL) inhibited cell viability in a dose-dependent manner (Figure 1C). EGCG (60 μg/mL) inhibited PANC-1 cell viability by 69% (Figure 1D). Y-27632 (20 μM) inhibited PANC-1 cell viability by 17% (Figure 1D), and 60 μg/mL EGCG + 20 μM Y-27632 inhibited PANC-1 cell viability by 82% (Figure 1D). These results suggest that 20 μM Y-27632 enhanced the sensitivity of PANC-1 cells to 60 μg/mL EGCG and suppressed cell proliferation.

Y-27632 improved the anti-migration and apoptosis effect of EGCG in PANC-1 cells

The effect of 60 μg/mL EGCG on the PANC-1 cell migration in the presence of 20 μM Y-27632 was evaluated using transwell migration assays (Figure 2A). Following treatment with 60 μg/mL EGCG + 20 μM Y-27632, the cell migration significantly decreased compared with that of the untreated PANC-1 control and the cells treated with 20 μM Y-27632 or 60 μg/mL EGCG alone (Figure 2B). These data suggest that PANC-1 cell migration is inhibited by treatment with 60 μg/mL EGCG + 20 μM Y-27632. Under Hoechst 33258 staining (Figure 2C), the normal PANC-1 cells without 60 μg/mL EGCG and 20 μM Y-27632 intervention were dark blue (control), but early apoptotic nuclei due to chromosomal collapse were stained bright blue in the 60 μg/mL EGCG and 20 μM Y-27632 treatment groups (Figure 2C), and were easily distinguished from the normal cells. Also, the number of bright blue-stained nuclei

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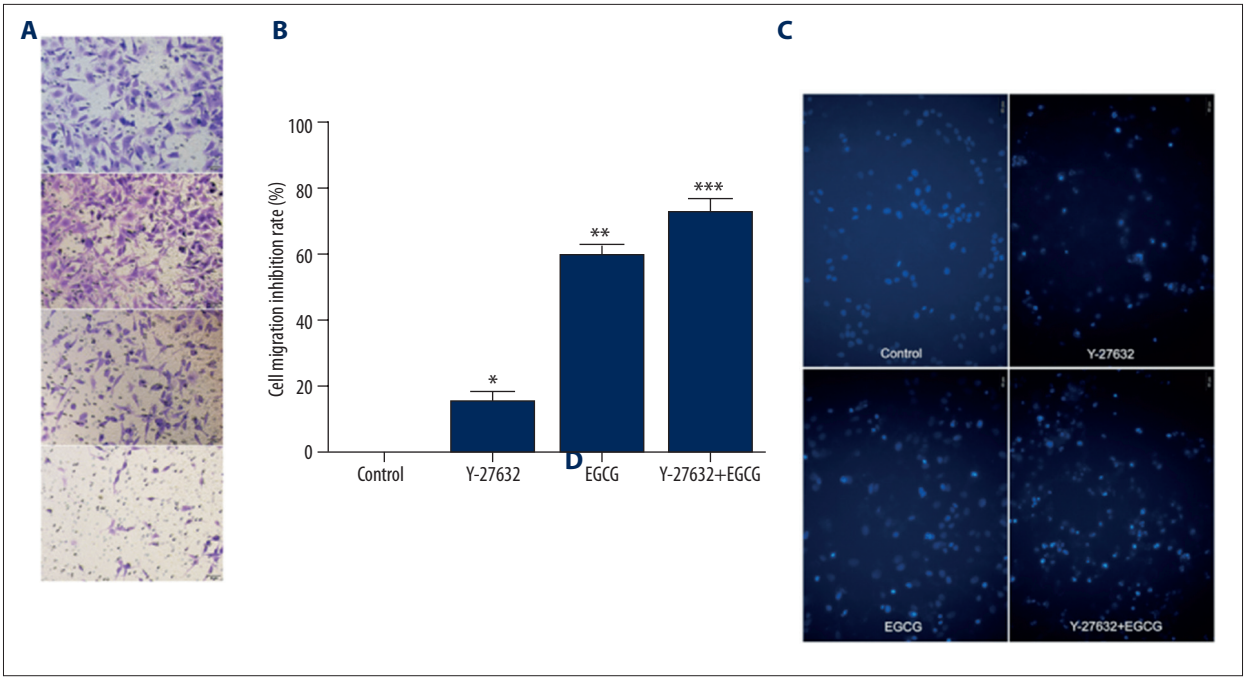

Figure 2. Effect of the combination of EGCG and Y-27632 on PANC-1 cell migration and apoptosis. PANC-1 cells were treated separately with DMSO (control), 60 μg/mL EGCG, 20 μM Y-27632, and 60 μg/mL EGCG + 20 μM Y-27632 for 48 h. (**A**) The effect of 60 μg/mL EGCG on the PANC-1 cell migration in the presence of 20 μM Y-27632 was evaluated using transwell migration assays. (**B**) Data represent mean ± SEM, *n*=3. Compared with control, * *P*<0.05, ** *P*<0.01. Compared with control, Y-27632, or EGCG, *** *P*<0.01. (**C**) Apoptosis of EGCG and Y-27632 in PANC-1 cells was determined by Hoechst 33258 staining.

increased (Figure 2C) in the 60 μg/mL EGCG + 20 μM Y-27632 treatment group.

Combination of Y-27632 and EGCG increased the expression of PPARα mRNA and caspase-3 mRNA

The expression of PPARα *mRNA* and caspase-3 *mRNA* was determined by RT-qPCR. The amplification and melting curves of PPARα and caspase-3 are shown in Figure 3A, 3B. Significant changes in the expression of PPARα *mRNA* and caspase-3 *mRNA* were observed in PANC-1 cells treated with 60 μg/mL EGCG or 20 μM Y-27632 alone, and 60 μg/mL EGCG + 20 μM Y-27632. Treatment with 20 μM Y-27632 + 60 μg/mL EGCG caused a sharp increase in the expression of PPARα *mRNA* and caspase-3 *mRNA* compared with the levels detected following treatment with 60 μg/mL EGCG or 20 μM Y-27632 alone (Figure 3C).

Discussion

Our study demonstrated that Y-27632 sensitized the PANC-1 cells to the inhibitory effects of EGCG on cell proliferation and migration. Furthermore, the combination of Y-27632 and EGCG promoted apoptosis of the PANC-1 cells. The results also indicate that the Y-27632-induced sensitization is related to the increased expression of PPARα mRNA and caspase-3 mRNA.

This study, using the CCK-8 assay, evaluated the probable effect of different concentrations of EGCG (20, 40, 60, and 80 μg/mL) on PANC-1 cells. The results are in agreement with a previous study [25], which showed that EGCG (20–80 μg/mL) inhibited the proliferation of PANC-1 cells in a dose-dependent manner. The results also showed that at 48 h, Y-27632 enhanced the sensitivity of PANC-1 cells to EGCG and suppressed the proliferation of PANC-1 cells.

In the present study, using transwell migration assays and Hoechst 33258 staining, the effect of 20 μM Y-27632 combined with 60 μg/mL EGCG on the PANC-1 cell migration and apoptosis was evaluated. The results also showed that 20 μM Y-27632 enhanced the anti-migration effect of 60 μg/mL EGCG on PANC-1 cells when treated for 48 h. Furthermore, the effects of 60 μg/mL EGCG in regulating apoptosis of PANC-1 cells enhanced when treated with 20 μM Y-27632 + 60 μg/mL EGCG.

Tumor growth and metastasis depend on angiogenesis [26,27], and gene expression profiling of PPARα has been used in several studies [28,29], but a very few studies included pancreatic cancer. When PANC-1 cells were exposed to EGCG [30], the

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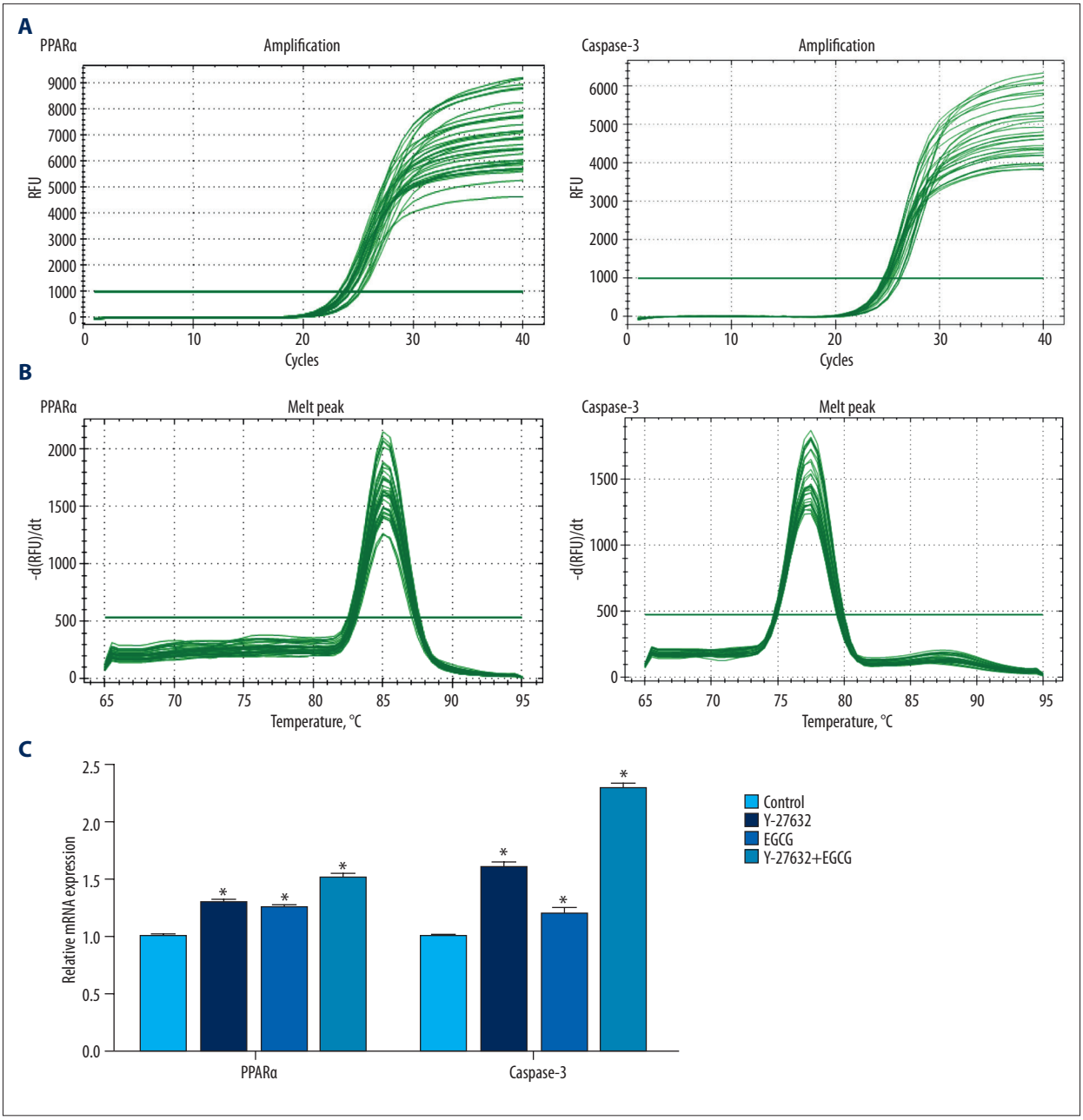

Figure 3. The combination of EGCG and Y-27632 increased the expression of PPARα *mRNA* and caspase-3 *mRNA*. PANC-1 cells were treated with DMSO (control), EGCG (60 μg/ml EGCG), or Y-27632 (20 μM Y-27632), and EGCG combined with Y-27632 (60 μg/ml EGCG + 20 μM Y-27632) for 48 h. The expression of PPARα *mRNA* and caspase-3 *mRNA* was analyzed by qRT-PCR. (**A**) The amplification curves of PPARα and caspase-3. (**B**) The melting curves of PPARα and caspase-3. (**C**) The relative gene expression of PPARα *mRNA* and caspase-3 *mRNA* in each group. Data represent mean ± SEM, *n*=3. Treatment with 20 μM Y-27632 + 60 μg/mL EGCG (compared with control, * *P*<0.05) caused a sharp increase in the expression of PPARα mRNA and caspase-3 *mRNA* compared with the levels detected following treatment with 60 μg/mL EGCG or 20 μM Y-27632 alone (compared with 20 μM Y-27632 + 60 μg/mL EGCG, * *P*<0.05).

expression of PPARα, a direct negative regulator of heme oxygenase (HO-1) activation by EGCG [31], which confers cell susceptibility to EGCG, increased at the protein level in a dose-dependent manner. EGCG induces apoptosis and inhibits the growth of PANC-1 tumors [32] and activates caspase-3 is a dose-dependent manner. Therefore, the capacity of Y-27632 to sensitize PANC-1 cells to EGCG by activated PPARα mRNA and caspase-3 mRNA expression were investigated.

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Conclusions

The combination of EGCG and Y-27632 significantly increased the expression of PPARα mRNA and caspase-3 mRNA in PANC-1 cells. These data suggest that Y-27632 sensitizes PANC-1 cells to EGCG by increasing the expression of PPARα mRNA and caspase-3 mRNA. The synergistic effect of the combination of EGCG and Y-27632 on PANC-1 provides new and useful information for its application in pancreatic cancer therapy.

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References:

1. Herreros-Villanueva M, Hijona E, Cosme A, Bujanda L: Mouse models of pancreatic cancer. World J Gastroenterol, 2012; 18: 1286–94
2. Gao J, Yuan S, Jin J et al: PPARalpha regulates tumor progression, foe or friend? Eur J Pharmacol, 2015; 765: 560–64
3. Sun Y, Zhang L, Gu HF et al: Peroxisome proliferator-activated receptor-alpha regulates the expression of pancreatic/duodenal homeobox-1 in rat insulinoma (INS-1) cells and ameliorates glucose-induced insulin secretion impaired by palmitate. Endocrinology, 2008; 149: 662–71
4. Bishop-Bailey D: PPARs and angiogenesis. Biochem Soc Trans, 2011; 39: 1601–5
5. Birame BM, Jigui W, Fuxian Y et al: Potentiation of apoptin-induced apoptosis by cecropin B-like antibacterial peptide ABPs1 in human HeLa cervical cancer cell lines is associated with membrane pore formation and caspase-3 activation. J Microbiol Biotechnol, 2014; 24(6): 756–64
6. Hu Q, Peng J, Liu W: Elevated cleaved caspase-3 is associated with shortened overall survival in several cancer types. Int J Clin Exp Pathol, 2014; 7(8): 5057–70
7. Modernelli A, Naponelli V, Giovanna Troglio M et al: EGCG antagonizes Bortezomib cytotoxicity in prostate cancer cells by an autophagic mechanism. Sci Rep, 2015; 5: 15270
6. Tian LW, Tao MK, Xu M et al: Carboxymethyl- and carboxyl-catechins from ripe Pu-er tea. J Agric Food Chem, 2014; 62: 12229–34
7. Dou QP: Molecular mechanisms of green tea polyphenols. Nutr Cancer, 2009; 61: 827–35
8. Isler D, Ozaslan M, Karagoz ID et al: Antitumoral effect of a selective Rho-kinase inhibitor Y-27632 against Ehrlich ascites carcinoma in mice. Pharmacol Rep, 2014; 66: 114–20
9. Kolluru GK, Majumder S, Chatterjee S: Rho-kinase as a therapeutic target in vascular diseases: Striking nitric oxide signaling. Nitric Oxide, 2014; 43: 45–54
10. Schaafsma D, Bos IS, Zuidhof AB et al: The inhaled Rho kinase inhibitor Y-27632 protects against allergen-induced acute bronchoconstriction, airway hyperresponsiveness, and inflammation. Am J Physiol Lung Cell Mol Physiol, 2008; 295: L214–19
11. Nakashima M, Adachi S, Yasuda I et al: Inhibition of Rho-associated coiled-coil containing protein kinase enhances the activation of epidermal growth factor receptor in pancreatic cancer cells. Mol Cancer, 2011; 10: 79
12. Gogebakan B, Bayraktar R, Suner A et al: Do fasudil and Y-27632 affect the level of transient receptor potential (TRP) gene expressions in breast cancer cell lines? Tumour Biol, 2014; 35: 8033–41
13. Huang HP, Wang CJ, Tsai JP et al: Y27632 attenuates the aristolochic acid-promoted invasion and migration of human urothelial cancer TSGH cells *in vitro* and inhibits the growth of xenografts *in vivo*. Nephrol Dial Transplant, 2012; 27: 565–75
14. Routhier A, Astuccio M, Lahey D et al: Pharmacological inhibition of Rho-kinase signaling with Y-27632 blocks melanoma tumor growth. Oncol Rep, 2010; 23: 861–67
15. Bimonte S, Leongito M, Barbieri A et al: Inhibitory effect of (–)-epigallocatechin-3-gallate and bleomycin on human pancreatic cancer MiaPaca-2 cell growth. Infect Agent Cancer, 2015; 10: 22
16. Hodges V, Tucci M, Benghuzzi H: The effects of metformin and EGCG on PANC-1 cell survival. Biomed Sci Instrum, 2015; 51: 393–99
17. Sun L, Zhang C, Li P: Copolymeric micelles for delivery of EGCG and cyclopamine to pancreatic cancer cells. Nutr Cancer, 2014; 66(5): 896–903
15. He XJ, Jiang XT, Ma YY et al: REG4 contributes to the invasiveness of pancreatic cancer by upregulating MMP-7 and MMP-9. Cancer Sci, 2012; 103: 2082–91
16. Chen A, Huang X, Xue Z et al: The role of p21 in apoptosis, proliferation, cell cycle arrest, and antioxidant activity in UVB-irradiated human HaCaT keratinocytes. Med Sci Monit Basic Res, 2015; 21: 86–95
17. Chen Z, Yang Y, Liu B et al: Promotion of metastasis-associated gene expression in survived PANC-1 cells following trichostatin a treatment. Anticancer Agents Med Chem, 2015; 15: 1317–25
18. Zhang HJ, Yao DF, Yao M et al: Annexin A2 silencing inhibits invasion, migration, and tumorigenic potential of hepatoma cells. World J Gastroenterol, 2013; 19: 3792–801
19. Zeng T, Zhang CL, Song FY et al: CMZ reversed chronic ethanol-induced disturbance of PPAR-alpha possibly by suppressing oxidative stress and PGC-1alpha acetylation, and activating the MAPK and GSK3beta pathway. PLoS One, 2014; 9: e98658
20. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 2001; 25: 402–8
21. Muller PY, Janovjak H, Miserez AR, Dobbie Z: Processing of gene expression data generated by quantitative real-time RT-PCR. Biotechniques, 2002; 32: 1372–74, 1376, 1378–79
22. Wang HM, Lu JH, Chen WY, Gu AQ: Upregulated lncRNA-UCA1 contributes to progression of lung cancer and is closely related to clinical diagnosis as a predictive biomarker in plasma. Int J Clin Exp Med, 2015; 8: 11824–30
23. Zhang QL, Wang L, Zhang YW et al: The proteasome inhibitor bortezomib interacts synergistically with the histone deacetylase inhibitor suberoylanilide hydroxamic acid to induce T-leukemia/lymphoma cells apoptosis. Leukemia, 2009; 23: 1507–14
24. Chu R, van Hasselt A, Vlantis AC et al: The cross-talk between estrogen receptor and peroxisome proliferator-activated receptor gamma in thyroid cancer. Cancer, 2014; 120: 142–53
25. Guo R, Li W, Liu B et al: Resveratrol protects vascular smooth muscle cells against high glucose-induced oxidative stress and cell proliferation *in vitro*. Med Sci Monit Basic Res, 2014; 20: 82–92
26. Yildirim V, Doganci S, Yesildal F et al: Sodium nitrite provides angiogenic and proliferative effects *in vivo* and *in vitro*. Med Sci Monit Basic Res, 2015; 21: 41–46
27. Fong P, Meng LR: Effect of mTOR inhibitors in nude mice with endometrial carcinoma and variable PTEN expression status. Med Sci Monit Basic Res, 2014; 20: 146–52
28. Takizawa Y, Nakata R, Fukuhara K et al: The 4'-hydroxyl group of resveratrol is functionally important for direct activation of PPARalpha. PLoS One, 2015; 10: e0120865
29. Thomas M, Bayha C, Klein K et al: The truncated splice variant of peroxisome proliferator-activated receptor alpha, PPARalpha-tr, autonomously regulates proliferative and pro-inflammatory genes. BMC Cancer, 2015; 15: 488
30. Luo J, Xue J, Ge X et al: Mechanism of sensitizing effect of PPARα activation on epigallocatechin-3-gallate (EGCG) in cancer cells. Science & Technology Review, 2013; 31: 21–26
31. Šarić A, Sobočanec S, Mačak Šafranko Ž et al: Diminished resistance to hyperoxia in brains of reproductively senescent female CBA/H mice. Med Sci Monit Basic Res, 2015; 21: 191–99
32. Kostin SF, McDonald DE, McFadden DW: Inhibitory effects of (–)-epigallocatechin-3-gallate and pterostilbene on pancreatic cancer growth *in vitro*. J Surg Res, 2012; 177(2): 255–62

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