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Curcumin attenuates doxorubicin-induced cardiotoxicity via suppressing oxidative stress and preventing mitochondrial dysfunction mediated by $14\text{-}3\text{-}3\gamma$

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Supplementary materials

Fig. S1. Effects of Cur, pAD/14-3-3γ-shRNA, pAD/scrRNAi, or a combination of Cur with pAD/14-3-3γ-shRNA or pAD/scrRNAi on cell viability of cardiomyocytes unexposed to Dox, and effects of pretreatment with pAD/14-3-3γ-shRNA or pAD/scrRNAi on cell viability of cardiomyocytes exposed to Dox. Cell viability did not change when using Cur alone (10 μM), pAD/14-3-3γ-shRNA alone, pAD/scrRNAi alone, Cur+pAD/14-3-3γ-shRNA, and Cur+pAD/scrRNAi when compared with the control group (p>0.05), or by using pAD/scrRNAi+Dox compared with Dox group (p>0.05). However, the cell viability of pAD/14-3-3γ-shRNA+Dox was lower when compared to that of Dox alone (p<0.01), indicating that 14-3-3γ could alleviate Doxinduced injury of cardiomyocytes. Values are expressed as the mean±SEM for eight individual experiments, **p<0.01 vs control group: $^{\triangle} _{p}$ <0.01 vs Dox group.

Fig. S2. Effects of Cur, pAD/14-3-3 γ -shRNA, pAD/scrRNAi, or a combination of Cur with pAD/14-3-3 γ -shRNA or pAD/scrRNAi on LDH activities in culture media unexposed to Dox, and effects of pretreatment with pAD/14-3-3 γ -shRNA or pAD/scrRNAi on LDH activities in culture mediums exposed to Dox. The LDH activity did not change when using Cur alone (10 μ M), pAD/14-3-3 γ -shRNA alone, pAD/scrRNAi alone, Cur+pAD/14-3-3 γ -shRNA, and Cur+pAD/scrRNAi when compared with the control group (p>0.05), or by using pAD/scrRNAi+Dox when compared with the Dox group (p>0.05). However, the LDH activity of pAD/14-3-3 γ -shRNA+Dox was higher when compared to that of Dox alone (p<0.01). Values are expressed as the mean±SEM for eight individual experiments. **p<0.01, vs control group: $^{\triangle} p$ <0.01 vs Dox group.

Fig. S3. Effects of Cur, pAD/14-3-3γ-shRNA, pAD/scrRNAi, or a combination of Cur with pAD/14-3-3y-shRNA or pAD/scrRNAi on the expression of 14-3-3y of cardiomyocytes unexposed to Dox, and effects of pretreatment with pAD/14-3-3yshRNA or pAD/scr RNAi on the expression of 14-3-3y of the cardiomyocytes **exposed to Dox.** The expression of 14-3-3γ in cardiomyocytes was upregulated using Cur alone (10 µM) and Cur+pAD/scrRNAi when compared with the control group (p<0.01). No changes were observed using pAD/scrRNAi+Dox when compared with the Dox group (p>0.05) or by using pAD/scrRNAi alone compared with the control group (p>0.05). However, the expression of 14-3-3 γ was significantly downregulated using pAD/14-3-3y-shRNA alone, Cur+pAD/14-3-3y-shRNA, and pAD/14-3-3y-sh RNA+Dox when compared to that of the control group. From left to right, lane 1: Control; lane 2: Cur alone; lane 3: pAD/14-3-3y-shRNA alone; lane 4: pAD/scrRNAi alone; lane 5: Cur+pAD/14-3-3γ-shRNA; lane 6: Cur+pADscr RNAi; lane 7: Dox; lane 8: pAD/14-3-3γ-shRNA+Dox; lane 9: pAD/scrRNAi+Dox. Values are expressed as fold changes over the level of β-actin and are presented as the mean±SEM from five individual experiments. **p<0.01, vs control group; $^{\blacktriangle}p$ <0.01 vs Dox group.

Fig. S4. Effects of Cur, pAD/14-3-3γ-shRNA, pAD/scrRNAi, or a combination of Cur with pAD/14-3-3γ-shRNA or pAD/scrRNAi on the expression of p-Bad (S112) of cardiomyocytes unexposed to Dox, and effects of pretreatment with pAD/14-3 -3γ-shRNA or pAD/scrRNAi on the expression of p-Bad(S112) of cardiomyocytes exposed to Dox. The expression of p-Bad(S112) in cardiomyocytes was upregulated using Cur alone (10 μM) and Cur+pAD/scrRNAi when compared with the control

group (p<0.01). No changes were observed using pAD/scrRNAi+Dox when compared with the Dox group (p>0.05) or by using pAD/scrRNAi alone compared with the control group (p>0.05). However, the expression of p-Bad(S112) was significantly downregulated using pAD/14-3-3γ-shRNA alone, Cur+pAD/14-3-3γ-shRNA, and pAD /14-3-3γ-shRNA+Dox when compared to that of the control group. From left to right, lane 1: Control; lane 2: Cur alone; lane 3: pAD/14-3-3γ-shRNA alone; lane 4: pAD/scr RNAi alone; lane 5: Cur+pAD/14-3-3γ-shRNA; lane 6: Cur+pADscrRNAi; lane 7: Dox; lane 8: pAD/14-3-3γ-shRNA+Dox; lane 9: pAD/scrRNAi+Dox. Values are expressed as fold changes over the level of β-actin and are presented as the mean±SEM from five individual experiments. **p<0.01, vs control group; $^{\blacktriangle}$ p<0.01 vs Dox group.

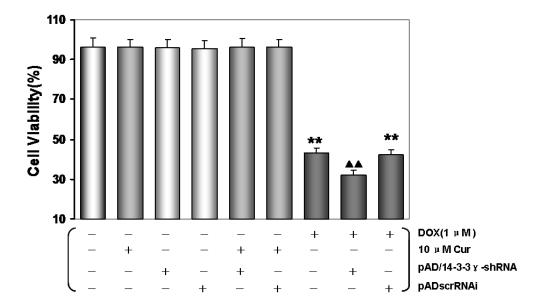


Fig. S1

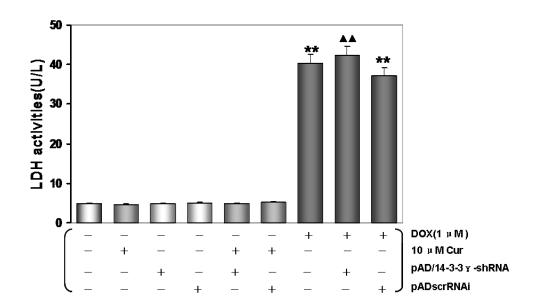


Fig. S2

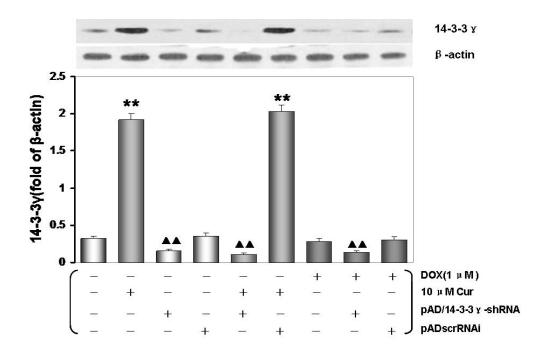


Fig. S3

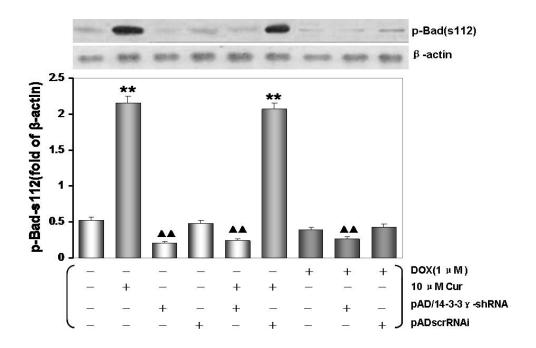


Fig. S4