H9c2 cardiomyoblast cell apoptosis induced by hypoxia was fully rescued by Tetramethylpyrazine via up-regulated PI3K/Akt survival pathway

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Abstract

In Taiwan, trauma has the highest mortality rate in the population below 40 years old of Taiwan, and head injury and hemorrhagic shock (HS) in early time, organ failure in later period, are the major death causes of trauma. However, HS may lead sequentially to hemodynamic instability, decreases in oxygen delivery, decreased tissue perfusion, cellular hypoxia, organ damage, and death. Our previous findings HS has the dominant positive influence in cardiac apoptosis pathway. All evidences even demonstrated the diabetic rat under trauma-induced HS, synergistically causes the myocardial cell damage. Previously, we demonstrated that Chuan Xiong will keep to totally rescue the synergistic H9c2 cardiomyoblast cell injury in high-glucose (HG) enhanced by hypoxia-induced HS. One of the most important active ingredients of Chuan Xiong is Tetramethylpyrazine (TMP), which is reported that TMP significantly elevated the survival rate in ischemic brain injury. Several studies indicated that TMP prevents inducible NO synthase expression to anti-inflammation and against cell damage in different kinds of cell types. Therefore we further to investigated if TMP could against hypoxic (<1% oxygen) condition in H9c2 cells for 24 hrs. Our results showed the hypoxia caused hypoxia related proteins HIF-1α, BNIP3 and IGFBP3 were highly increasing, and pro-apoptotic protein Bak were also increased, and up regulate downstream Caspase 9 and 3 result in cell death, all phenomena fully recovered after TMP treatment. We observed TMP cuold also up-regulated IGF1 receptor survival pathway, and enhance PI3K/Akt pathway. However, once PI3K was been blocked by specific si-RNA, Caspase 3 could not be decreased after TMP treatment. The protective effect of TMP was via enhanced survival pathway in H9c2 cardiomoblast cells. We intend to use this model to identify the TMP could restore the cardiac hypoxic damage caused by hemorrhagic shock.

Result

Fig. 1

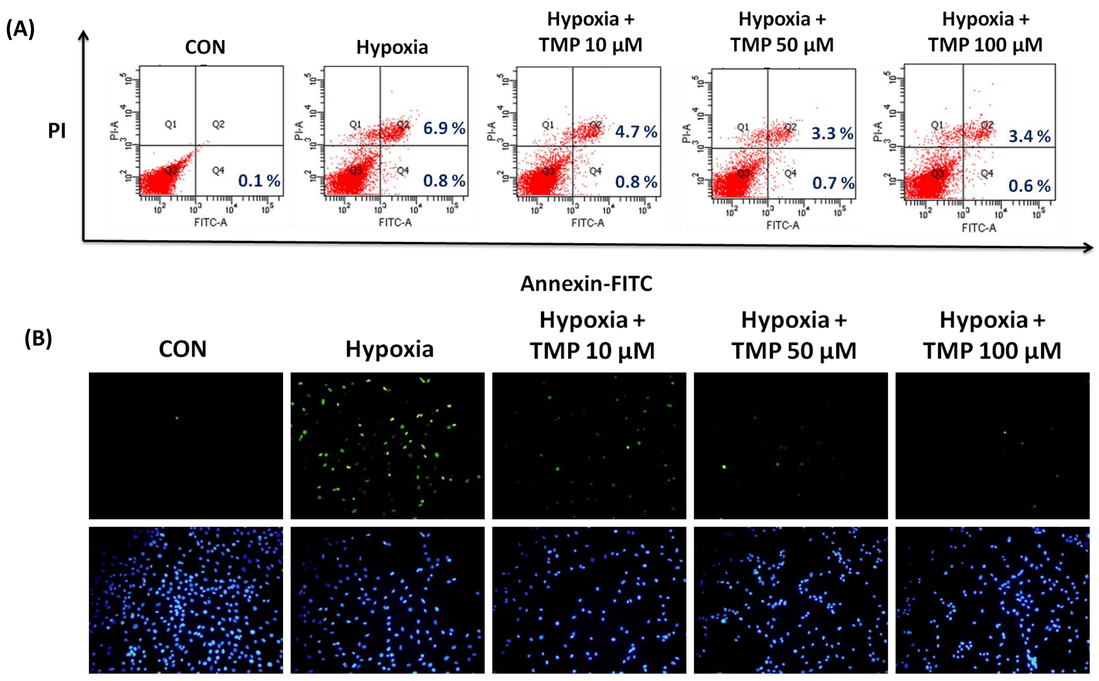

Figure 1 The effect of TMP on hypoxia-induced H9c2 cells apoptosis.
H9c2 cells were subjected to hypoxia (< 1% O2) environment and co-treated with TMP (10, 50, 100 μM) for 24 hrs. (A) The cells were harvested and staining with Annexin V-FITC and PI, then analyzed by flow cytometry. Apoptotic cells were calculated as lower right quadrants. (B) DAPI staining (Blue) spots in the left pannel represent cell nuclei. TUNEL stating (green) spots in the right pannel represent apoptotic bodies.

Fig. 2

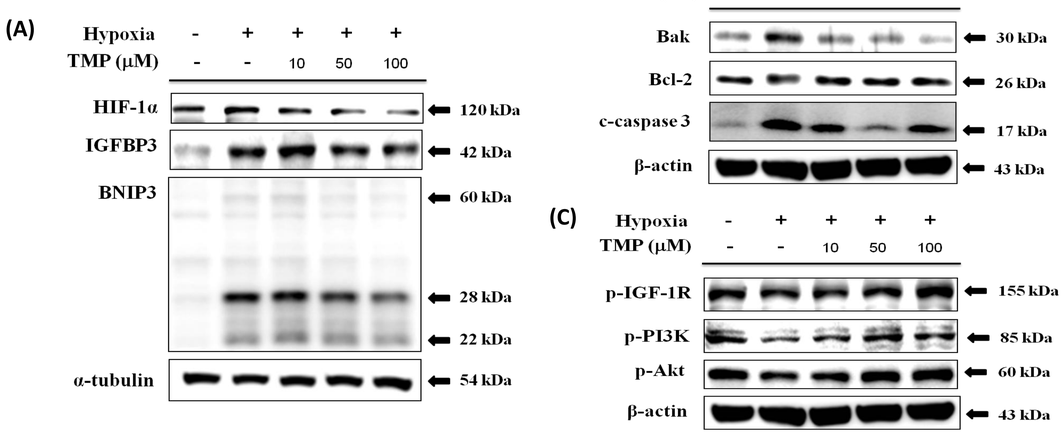

Figure 2 Tetramethylpyrazine (TMP) suppressed the hypoxia related proteins and apoptotic proteins induced by hypoxia in H9c2 cells.
H9c2 cells were co-treated with various concentrations of TMP (10, 50, 100 μM) for 24 hrs incubated with hypoxia, Entire protein extract from cells was separated using 10% SDS-PAGE, transferred to PVDF membranes, and immunoblotted with antibodies against (A) HIF-1α, BNIP3, and IGFBP3, (B) p-IGF-1 Receptor, p-PI3K, and p-Akt , (C) Bax, Bcl-2, and cleaved caspase-3. Equal loading was verified with an anti-α-tubulin or anti-β-actin.

Fig. 3

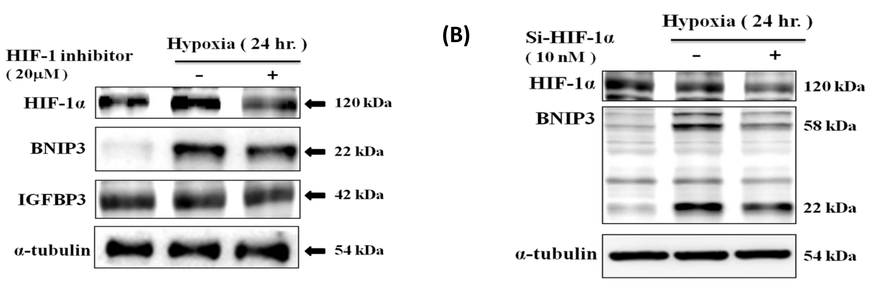

Figure 3 Identify the role of HIF-1α on hypoxia-related downstream proteins IGFBP3 and BNIP3 in hypoxia-induced H9c2 cardiomyoblast cells
H9c2 cells were exposed to hypoxia environment and treated with (A) HIF-1 inhibitor (20μM) and (B) HIF-1α si-RNA 10nM for 24 hr, after treatment then harvested. Whole protein extract was analyzed by Western blotting and antibodies against IGFBP3, BNIP3, and HIF-1α..

Fig. 4

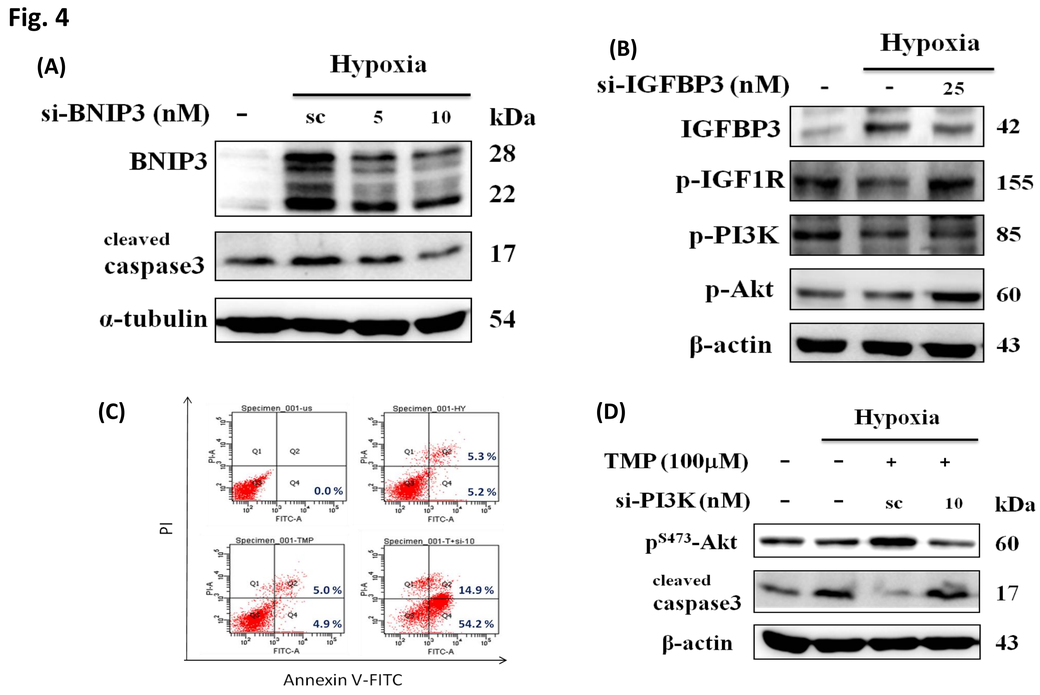

Figure 4 Identify the role of IGFBP3 and BNIP3 in hypoxia-induced H9c2 cardiomyoblast cells and TMP recued cell apoptosis via up regulated p-Akt
H9c2 cells were exposed to hypoxia environment and treated with si-RNA (A) BNIP3 (5nM and 10nM) for 24 hr, (B) IGFBP3 (25nM) for 48 hr. Whole protein extract was analyzed by Western blotting and antibodies against IGFBP3, BNIP3, cleaved caspase-3, and p-Akt. (C) co-treat with TMP 100 μM and si-PI3K 10nM for 24 hr and then harvested. The cells were harvested and staining with Annexin V-FITC and PI, then analyzed by flow cytometry. Apoptotic cells were calculated as lower right quadrants. (D) Whole protein extract was analyzed by Western blotting and antibodies against cleaved caspase-3, and p-Akt.

Fig. 5

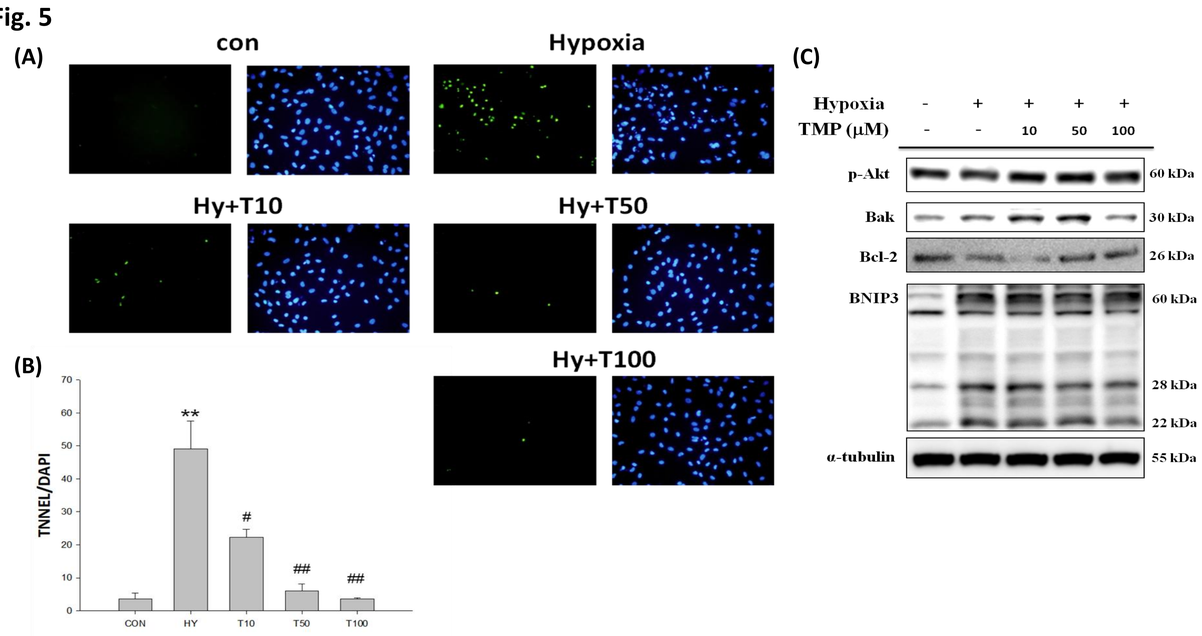

Figure 5 TMP inhibited hypoxia-induced cell death in neonatal cardiomyocytes
Neonatal primary cardiomyocytes were subjected to hypoxia (< 1% O2) environment and co-treated with various concentrations TMP (10, 50, 100 μM) for 24 hrs. (A) cells were analyzed by TUNEL assay to represent apoptotic bodies. (B) Bars represent the percentage of TUNEL-positive cells based on total stained cells by DAPI. The results are expressed as mean ±S.D. of three independent determinations. **p <0.01, ***p <0.001 as compared with the hypoxia group, ## p<0.01 , ### p<0.001 as compared with the control group. (C) After treatment, whole protein extract was analyzed by Western blotting and antibodies against Bcl-2, Bax, BNIP3, and p-Akt.

Fig. 6

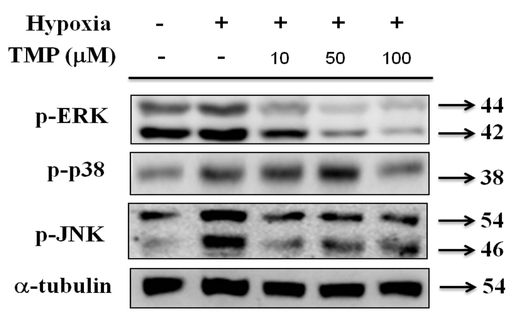

Figure 6 Effect of TMP on MAPK pathway induced by hypoxia in H9c2 cells
H9c2 cells were exposed to hypoxia environment were co-treated with hypoxia and TMP for 24 hrs, and antibodies against p-p38, p-ERK1/2, and p-JNK1/2. Equal loading was verified with an anti-α-tubulin or anti-β-actin.

Conclusion

Fig. 7

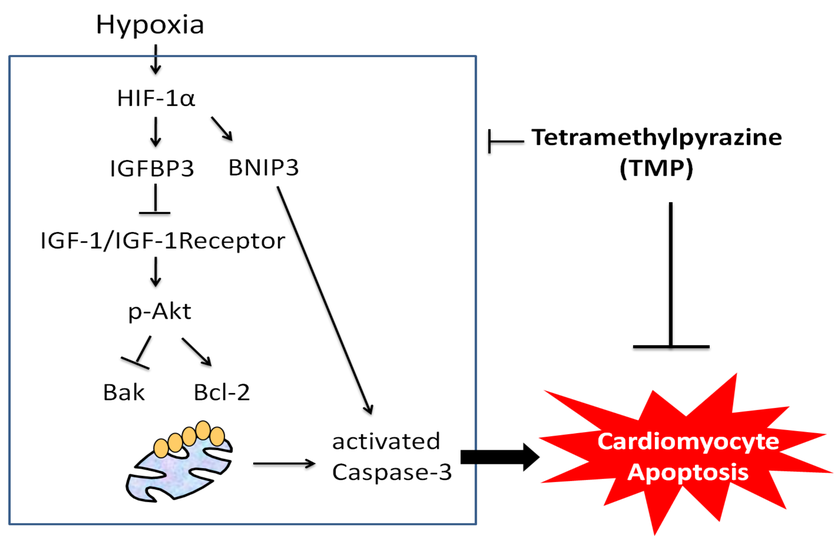

Figure 7 Proposed mechanism for TMP protected against hypoxia-induced cardiac apoptosis.
Our proposed hypothesis is that TMP protects hypoxia-induced H9c2 cardiomyocyte cell apoptosis by inhibited IGFBP3 to enhance p-IGF1R survival pathway, and up regulated p-Akt . TMP can also inhibited the activation of Bax, and Caspase-3 apoptotic related proteins to reduced hypoxia-induced cardiomyocyte cell death.