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A newly synthesized Ligustrazine stilbene derivative inhibits PDGF-BB induced vascular smooth muscle cell phenotypic switch and proliferation via delaying cell cycle progression

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ABSTRACT

Vascular Smooth muscle cells (VSMCs) possess remarkable phenotype plasticity that allows it to rapidly adapt to fluctuating environmental cues, including the period of development and progression of vascular diseases such as atherosclerosis and restenosis subsequent to vein grafting or coronary intervention. Although VSMC phenotypic switch is an attractive target, there is no effective drug so far. Using rat aortic VSMCs, we investigate the effects of Ligustrazine and its synthetic derivatives on platelet-derived growth factor-BB (PDGF-BB) induced proliferation and phenotypic switch by a cell image-based screening of 60 Ligustrazine stilbene derivatives. We showed that one of the Ligustrazine stilbene derivatives TMP-C_{4a} markedly inhibited PDGF-BB-induced VSMCs proliferation in a time and dose-dependent manner, which is more potent than Ligustrazine. Stimulation of contractile VSMCs with PDGF-BB significantly reduced the contractile marker protein α-smooth muscle actin expression and increased the synthetic marker proteins osteopontin expression. However, TMP-C_{4a} effectively reversed this phenotypic switch, which was accompanied by a decreased expression of Matrix metalloproteinase 2 and 9 (MMP2 and MMP9) and cell cycle related proteins, including cyclin D1 and CDK4. In conclusion, the present study showed that a new Ligustrazine stilbene derivative TMP-C_{4a} suppressed PDGF-induced VSMC proliferation and phenotypic switch, indicating that it has a potential to become a promising therapeutic agent for treating VSMC-related atherosclerosis and restenosis.

1. Introduction

Vascular smooth muscle cells (VSMCs) modulate their phenotype from contractile to synthetic states in response to environmental changes (Song et al., 2016), which characterized an increased proliferative and migratory activities, loss of contractility, and abnormal extracellular matrix production (Osman et al., 2016). This modulation plays a crucial role in cardiovascular diseases such as atherosclerosis, restenosis after angioplasty, and hypertension (Newby and Zaltsman, 2000). VSMCs within the healthy adult blood vessel exhibit a differentiated phenotype characterized by expression of smooth muscle contractile genes such as α-smooth muscle actin (α-SMA), smooth muscle-myosin heavy chain (SMMHC) (Uranishi et al., 2001), smooth muscle 22α (SM22α), and smoothelin (Gomez and Owens, 2012). During vascular pathologies development, VSMCs become phenotypically modulated and the synthetic phenotype marker proteins, such as osteopontin (OPN) and vimentin (Scatena et al., 2007) are up-regulated (Chen et al., 2016).

A wide variety of signaling factors have been implicated in the transition to synthetic phenotype of VSMCs, including platelet-derived growth factor (PDGF)-BB (Kawai-Kowase and Owens, 2007), basic fibroblast growth factor (bFGF), insulin-like growth factors (IGFs), epidermal growth factor, a-thrombin, factor Xa, angiotensin II (AngII), endothelin-1, and unsaturated lysophosphatidic acids (Beamish et al., 2010). PDGF-BB is a member of the PDGF family and a critical mitogen for fibroblasts mesangial cells and VSMCs (Ha et al., 2015). It plays crucial roles in pathophysiological conditions, such as neoplasia and atherosclerosis (Heldin and Westermark, 1999).

Matrix metalloproteinase 2 and 9 (MMP2 and MMP9) are critical enzymes participating in extracellular matrix remodeling, as well as cell

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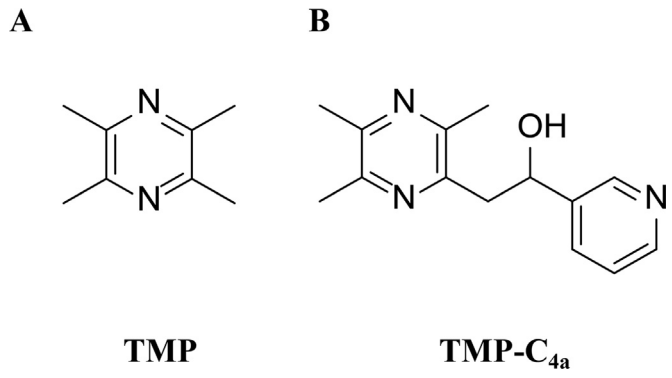

Fig. 1. Chemical structures of Tetramethylpyrazine (TMP) (A) and TMP-C_{4a} (B).

migration and proliferation (Seo et al., 2015). MMP2 expression in VSMC has been linked to several pathological situations, particularly in atherosclerotic plaques, suggesting a pathogenic role for MMP2 in the progression of atherosclerosis (Volcik et al., 2010). MMP9 is also demonstrated to be critical for the development of arterial lesions by regulating both proliferation and migration of VSMC (Ma et al., 2015).

Chuanxiong, a crude herbal drug isolated from the dried root or rhizome of Rhizoma Chuanxiong, is one of the important herbs for treating cardiovascular diseases in China for centuries (Li et al., 2016). Tetramethylpyrazine (TMP) (Fig. 1A), the main part of Chuanxiong, is important for treating angiocardiopathy (Ren et al., 2007). Guo M. et al. demonstrates the proliferation of rat VSMCs induced by PDGF and its dose-dependent inhibition by TMP (Guo et al., 2016). We have previously synthesized a series of Ligustrazine stilbene derivatives (Unpublished) and now screened 60 of these derivatives using a cell-based VSMC phenotypic switch/proliferation dual assay and identified the most potent compound as TMP-C_{4a}(1-(pyridin-3-yl)-2-(3,5,6-trimethylpyrazin-2-yl) ethanol) (Fig. 1B). We further characterized the detailed role of (TMP-C_{4a}) in the regulation of PDGF-induced VSMC proliferation and the underlying molecular mechanisms.

2. Materials and methods

2.1. Materials

Ligustrazine and Ligustrazine stilbene derivatives were prepared as described previously (Jiao, 2016). Recombinant rat PDGF-BB was purchased from R & D Systems (Minneapolis, MN, USA). Primary antibodies against α-smooth muscle actin (ab7817), Osteopontin (ab8448), MMP2 (ab92536), MMP9 (ab76003), CDK4 (ab108357), and Cyclin D1 (ab134175) were purchased from Abcam (Cambridge, MA, USA). β-actin (8457) were purchased from Cell Signaling Technology (Danvers, MA , USA). PI/RNase Staining Solution (CY2001-P) was purchased from Tianjin Sungene Biotech Co. Ltd (Tianjin, China).

2.2. Cell culture and treatments

Male Sprague-Dawley rats aged at three to four weeks old were used for VSMC isolation. VSMCs were isolated from their thoracic aortas as previously described (Gordon et al., 1986). The cells were cultured in 5% CO_2 at 37 °C. When cells were passaged to the third generation, the SMC specific marker a-SMA was used for immunofluorescence identification. Only the cells at passages 3–8 and purity above 90% were used in this study (Hewer et al., 2011). Cells were cultured in DMEM (Gibco,

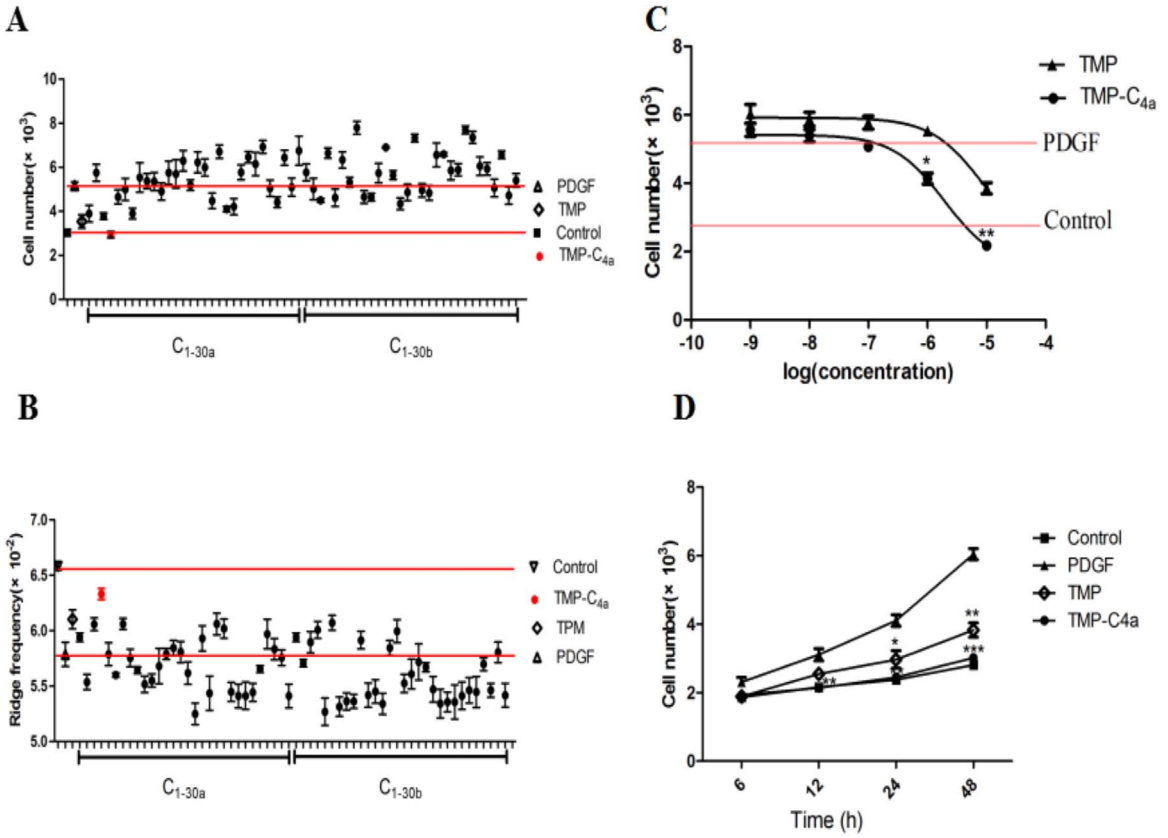

Fig. 2. High content screening of Ligustrazine stilbene derivatives of PDGF-stimulated VSMC proliferation and phenotypic switch. In the control group, VSMC were cultured with basal medium containing 1% serum for 48 h. In PDGF group, VSMCs were also cultured with basal medium but treated with PDGF-BB (20 ng/ml) for 48 h. (A) Screening based on cell numbers, VSMCs were treated with 10 μM TMP or Ligustrazine stilbene derivatives ($C_{1\text{-}30a}$, $C_{1\text{-}30b}$) and PDGF-BB (20 ng/ml) for 48 h, respectively. (B) Screening based on morphology change under the same condition with A. (C) VSMCs were treated with TMP (1×10^{-9}–10^{-5} M) or TMP-C_{4a} (1×10^{-9}–10^{-5} M) and PDGF-BB (20 ng/ml) for 48 h. (D) VSMCs were treated with 2 μM TMP or TMP-C_{4a} and PDGF-BB (20 ng/ml) for 6–48 h. *P < 0.05, **P < 0.01 compared with PDGF, n = 3.