Supplementary Materials

METHODS

RT-PCR

Total RNA was isolated from PASMC with TRIzol reagent and quantitated by NanoDrop (Thermo Scientific). 1 mg of total RNA was used for reverse transcription with Taq Man Reverse Transcription Reagents (Applied Biosystems), and the emerging first-strand cDNA was used as a template for PCR reaction with High Fidelity Master Mix (New England Biolabs). PCR primers were designed using the NCBI Primer Blast and synthesized by Integrated DNA Technologies. The primer sequences for human TAS2R (hTAS2R), rat TAS2R (rTAS2R) and mouse TAS2R (mTAS2R) are shown in Supplemental Tables 2, 3 and 4. RT-PCR results were resolved using 2% agarose gels and ethidium bromide staining.

Table S2. DNA primer sequences for the hTAS2Rs

Subtype	Forward (5'-3')	Reverse (5'-3')	Predicted amplicon size (bp)
Tas2r3	TCCTCTGGCTCAAGTGGAGA	TAAGGGAGGCAGGTACCACA	224
Tas2r4	GAATCCCCAGACGGAAGCTC	CCTGGAGAGTAAAGGGTGGC	171
Tas2r7	CCCTGCGGAGACATATCAGG	ACAGCTTTCAGGGCTCTCAC	91
Tas2r10	CCACAGCCATCTATCCCTGG	TCAATTGCTGCAGTACCCTCA	93
Tas2r14	TGGGTGGTGTCATAAAGAGCA	ACACACACCAGCTTCCGAAT	204
Tas2r39	TGTGGTCGGTCTGGCTTTTT	CTTTGATGGCCCCCATGTGA	173
Tas2r40	CCCACTGGTGACCGCATTAT	GGCAGCAAACCAGAGATTGG	180

Table S3. DNA primer sequences for the mTAS2Rs

Subtype	Forward (5'-3')	Reverse (5'-3')	Predicted amplicon size (bp)
Tas2r137	GCGCACTGCTCTTATCCTGT	CAGCAGAAGGTAGGCAACCA	203
Tas2r108	ATTTGTGTTTGCTGCCTCGG	ATCCGGAGAGGCAATTCTGC	115
Tas2r130	AGAACTCTACTGGCATGTGTGA	AGGGGAACAGCATGACCAAG	187
Tas2r110	AGCCTTGCAAACAGGGTTCT	GGCACCTCAGACAATGCAAC	221
Tas2r114	CCCAGTTGTTGCGAAGATGG	TTCCATCTGCCTGCGATGTC	196
Tas2r139	TAATGCCCTGGCTTCTGTGG	AGGAGGGAGTATTCCCGAAA	194
Tas2r140	CCAGCACCACAGCCCATATTA	TGGACTTCAGCCACCATACTG	243

Table S4. DNA primer sequences for the rTAS2Rs

Subtype	Forward (5'-3')	Reverse (5'-3')	Predicted amplicon size (bp)
Tas2r137	ATGTTGGGATTCACTGAAGGGATA	TGAGTCGTGGGTGTGGTAAG	234
Tas2r108	CGCTGCCTTGCTCTATTTCC	ATGTCCTGGAGGGTAAGCAG	94
Tas2r121	CAGAGGGGAACGAGACCCTA	ACCAGACAACTGGAATGCCT	237
Tas2r107	TACTGGGGAAACACGTCTTGG	CTCATGGCTTTCACATGAGC	212
Tas2r123	CACCATGGCCCACACAAAAG	TTTGGACCGGCACCTAAGAC	247
Tas2r139	AACGGTGATGCTGACTCCAG	ACCGGCAGACTGATTCTGAG	176
Tas2r144	GCAGCTTTATCCTGACCGCC	GGTAAAGTCGAACGTGGTGC	111

Transfection of HEK293

Twenty-four hours after plating, HEK293 cells at 60-80% confluency were transiently transfected with GFP, or TRPC6 expression plasmids using Lipofectamine 2000 transfection reagent, based on the manufacturer's protocol. Transfection was performed at 37°C in serum-free Opti-MEM medium (Gibco) with 0.5 μg/ml DNA and Transfection reagent (μl). After 4-6 hrs of incubation at 37°C with transfection media, the medium was changed to serum containing DMEM. $[Ca^{2+}]_{cyt}$ measurement was performed 48-72 hrs after the transient transfection. The cDNA vector used for transfecting the TRPC6 gene is pCMS-EGFP-rat-TRPC6 (8,481bp).

RESULTS

Table S1. The gene synonym between human, rat and mouse TAS2Rs

Human synonym	Rat synonym	Mouse synonym
Tas2r3	Tas2r137	Tas2r137
Tas2r4	Tas2r108	Tas2r108
Tas2r7	Tas2r121	Tas2r130
Tas2r10	Tas2r107	Tas2r110
Tas2r14	Tas2r123	Tas2r114
Tas2r39	Tas2r139	Tas2r139
Tas2r40	Tas2r144	Tas2r140

Table S5. Chloroquine inhibits the development of hypoxia-induced pulmonary hypertension in rats

	Normoxia	Normoxia+CHQ	Hypoxia	Hypoxia+CHQ
RVSP (mm Hg)	25.98±1.54	26.19±1.70	51.07±2.56*	32.83±3.05$^{\#}$
RV/(LV+S) (g/g)	0.32±0.013	0.30±0.012	0.54±0.026*	0.44±0.01$^{\#}$
RV/BW (mg/g)	0.64±0.03	0.71±0.03	1.14±0.08*	0.86±0.04$^{\#}$
HCT (%)	43.2±0.66	43.3±2.12	57.3±1.12*	50.9±0.86$^{\#}$
PA wall thickness (%)	24.8±1.25	23.6±1.15	52.8±2.90*	34.1±1.37$^{*\#}$

CHQ, Chloroquine; RVSP, right ventricular systolic pressure; RV, right ventricle; BW, body weight; HCT, hematocrit; PA, pulmonary artery. Data are means±SE, $^{*}P<0.05$ vs. Normoxia; $^{\#}P<0.05$ vs. Hypoxia. n=6 in each group.

Table S6. Chloroquine inhibits the progression of established pulmonary hypertension in Sugen5416/hypoxia rats model.

	Nor	**SuHx 3w**	**SuHx 3w+ NOR2w**	**SuHx 3w+ CHQ 2w**
RVSP (mm Hg)	25.01±1.14	64.15±5.48$^{*\#}$	101.86±2.62*	72.27±3.22$^{*\#}$
RV/(LV+S) (g/g)	0.30±0.021	0.56±0.022$^{*\#}$	0.72±0.047*	0.53±0.016$^{*\#}$
RV/BW (mg/g)	0.61±0.04	1.40±0.07$^{*\#}$	1.81±0.10*	1.26±0.03$^{*\#}$

CHQ, Chloroquine; SuHx, Sugen5416/hypoxia; Nor, Normoxia; RVSP, right ventricular systolic pressure; RV, right ventricle; BW, body weight. Data are means±SE, $^{*}P<0.05$ vs. Nor; $^{\#}P<0.05$ vs. SuHx 3w+Nor 2W. n=6 in each group.

Figure Legends

Figure S1. RT-PCR analysis of TAS2R in human PASMC (A), rat pulmonary arteries (B) and mouse pulmonary arteries. Transcripts of seven subtypes of hTAS2Rs (3, 4, 7, 10, 14, 39, 40) (A), rTAS2Rs (137, 108, 121, 107, 123, 139, 144) (B) and mTAS2Rs (137, 108, 130, 110, 114, 139,140) (C) are identified in in human PASMC, rat pulmonary arteries and mouse pulmonary arteries, respectively.

Figure S2. Chloroquine inhibits ATP-induced increase in $[Ca^{2+}]_{cyt}$ in HEK293 cells transiently transfected with the TRPC6 gene. *A*: Representative traces showing changes of $[Ca^{2+}]_{cyt}$ before and during extracellular application of ATP (100 μM) in HEK293 cells transfected with an empty vector (GFP) and the TRPC6 gene (*Trpc6*) in the absence (GFP, *Trpc6*) or presence (*Trpc6*+CHQ) of 200 μM chloroquine (CHQ). *B*: Summarized data (mean±SE) showing $[Ca^{2+}]_{cyt}$ before (Basal) and after (ATP) extracellular application of ATP in GFP- and *Trpc6*-transfected cells in the absence (GFP, *Trpc6*) or presence (*Trpc6*+CHQ) of chloroquine. **P*<0.05 vs. Basal. *C*: Summarized data (mean±SE) showing ATP-induced increases in $[Ca^{2+}]_{cyt}$ in GFP-transfected cells and *Trpc6*-transfected cells treated with (*Trpc6*+CHQ) or without (*Trpc6*) chloroquine. **P*<0.05 vs. Control; $^{\#}P$<0.05 vs. *Trpc6*.