

Upregulation of Human Prostaglandin Reductase 1 (PTGR1) Improves Efficacy of Hydroxymethylacylfulvene, an Anti-tumor Chemotherapeutic Agent

Xiang Yu, Melanie M. Erzinger, Kathryn E. Pietsch, Frances N. Cervoni-Curet, John Whang, John Niederhuber, Shana J. Sturla

Journal of Pharmacology and Experimental Therapeutics

Supplemental Figure 1. Primary sequence comparison of rPTGR1 and hPTGR1. The identical amino acids (83%) are represented in blue and categorically similar substitutions are denoted as “+”. The conserved NADPH binding motif is marked green.

Supplemental Figure 2. Expression and purification of recombinant FLAG-tagged hPTGR1. hPTGR1 was PCR cloned and ligated to pFLAG plasmid. The expression was performed in HEK293 cells and PTGR1 was affinity-purified from whole cell lysate. Lane 1-4 on the left panel indicates the input, pre-and post-incubation with FLAG antibody resin and first wash, respectively. Elution fraction 1 and 2 were immunoblotted against PTGR1 and FLAG antibody, respectively. Coomassie staining demonstrates that purity of the recombinant PTGR1 is high.

Supplemental Figure 3 Immunoblots of hPTGR1 in HCT15 transfected with pCep4-GFP or pCep4-hPTGR1 (a); SW620 and HepG2 cells treated with 20 μ M D3T (b).

JPET #195768

Supplemental Figure 4 Immunoblots of hPTGR1 in HepG2 and SW620 cells treated with 20 μ M D3T, 10, 20 μ M curcumin or 15, 40 μ M resveratrol.

human: 1 MVRTKTWTLKKHFVGYPTNSDFELKTSELPPLNNGEVILLEALFLTVDPYMRVAAKRLKEG
MV+ KTWTLKKHF G+PT+S+ FEL+T+ELPPL NGEVLEALFL+VDPYMRVAAK+LKEG

rat: 1 MVQAKTWTLKKHFEGFPTDSNFELRTTELPPLNNGEVILLEALFLSVDPYMRVAAKRLKEG

human: 61 DTMMGQQVAKVVESKNVALPKGTVLASPGWTTSHSISDGKDLEKLLTEWPDT I PLSLALG
D +MMG+QVA +VVESKN A P GTIV+A GWT+HSISDG L KL EWPD +PLSLALG

rat: 61 DSMMGEQVARVVESKNSAFPTGTIVVALLGWTSHSISDGNGLRKLPAEWPDKLPLSLALG

human: 121 TVGMPGLTAYFGLLEICGVKGETVMVNAAAGAVGSVVGQIAKLGCKVVGAVGSDEKVA
TVGMPGLTAYFGLL+ICG+KGGETV VNAAAGAVGSVVGQIAKLGCKVVG GSDEKVA

rat: 121 TVGMPGLTAYFGLLDICGLKGETV LVNAAAGAVGSVVGQIAKLGCKVVG TAGSDEKVA

human: 181 YLQKLGFDVVFNYKTVESLEETLKKASPDGYDCYFDNVGGFEFSNTVIGQMKKFGRIAICG
YL+KLGFDV FNYKTV+SLEE L+ ASPDGYDCYFDNVGGFEFSNTVI QMK FGRIAICG

rat: 181 YLKKLGFDVAFNYKTVKSLEEALRTASPDGYDCYFDNVGGFEFSNTVILQMKTFGRIAICG

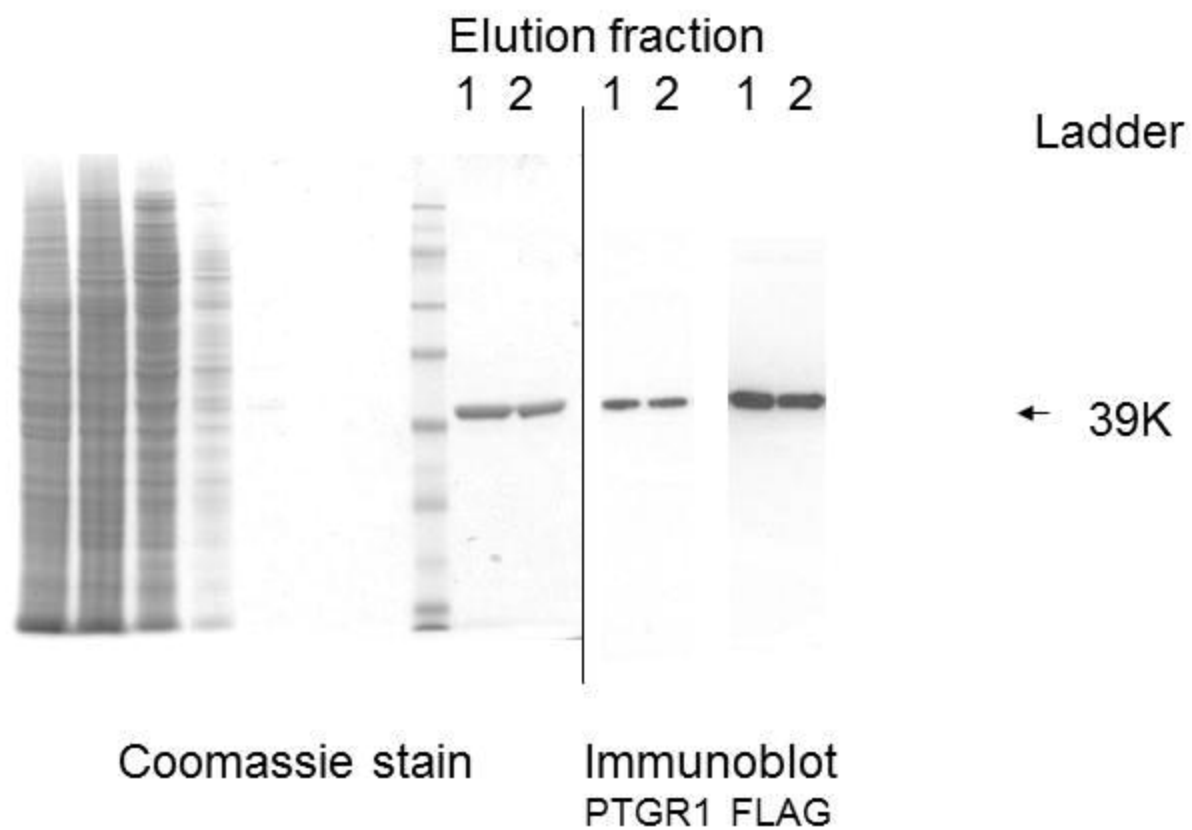
human: 241 AISTYNRTGPLPPGPPPE I VIYQELRMEAFVVYRWQGDARQKALKDLLKWV LEGKIYQYKE
AIS YNRTGP PPGP PE + +IYQ+LRME F+V RWQG+ RQKAL DL+ WV EGKI +Y E

rat: 241 AISQYNRTGPCPPGPSPEVI IYQLRMEGFIVTRWQGEVRQKA LTDLMNWWVSEGKIRYHE

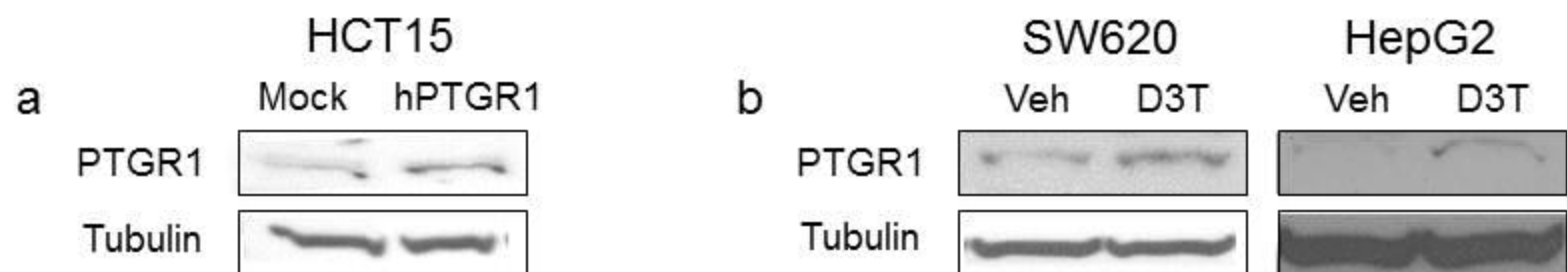
human: 301 YIEGFENMPAAFMGMLKGDNLGKTIVKA 329
YI EGFE MPAAFMGMLKGDNLGKTIVKA

rat: 301 YITEGFEEKMPAAFMGMLKGDNLGKTIVKA 329

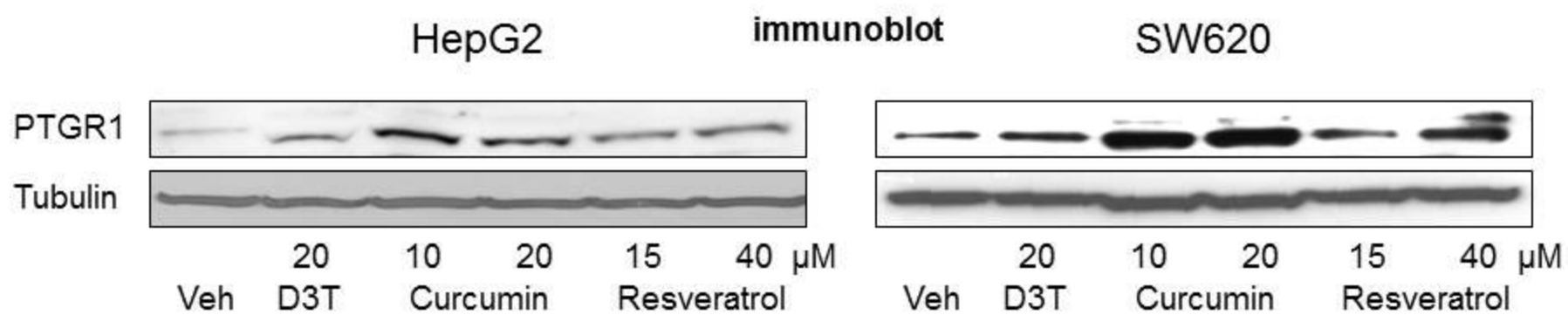
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4