Regulation of PPARγ activity by a novel serine phosphorylation

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Abstract

Mammalian ste20-like kinase (MST) signaling pathway is conserved from *Drosophila* to mammals and is essential for the proper regulation of organ growth, apoptosis and tumorigenesis. MST binds a scaffolding protein, SAV1 through the coiled-coil domain in the C-terminus and phosphorylates and activates SAV1. Previously, it was shown that MST2 and SAV1 interact with and activate peroxisome proliferator-activated receptor γ (PPARγ), a master regulator of adipogenesis. They were also shown to induce adipocyte differentiation. To further characterize this phenomenon, we sought to identify and analyze the phosphorylation of PPARγ by MST2 kinase. Using mass spectrometric analysis of PPARγ protein which was co-expressed with MST2 and SAV1 and immunoprecipitated, we detected a novel phosphorylation at Ser492 in C-terminal region of PPARγ protein that was co-expressed with MST2 and SAV1. A rabbit antiserum against PPARγ pSer492 was prepared and it detected a strong phosphorylation in PPARγ-WT but not in SA. Mutation of Ser492 to Ala (SA) resulted in a significant decrease in transcription activity of PPARγ compared to WT. Mutation of Ser492 to Glu (SE) increased the basal activity of PPARγ but had no effect on rosiglitazone-induced activation, indicating that PPARγ-SE is a partial phosphorylation-mimicking mutant. Interaction of PPARγ with PGC1β, one of PPARγ coactivators, was increased by rosiglitazone. However, SA mutant showed significantly inhibited interaction with PGC1β, indicating the identified Ser phosphorylation may be important for the binding of PPARγ to its coactivators. Finally, SA mutation significantly decreased PPARγ-induced adipocyte differentiation of 3T3-L1 cells. In this study, a novel Ser492 phosphorylation in PPARγ was presented and this phosphorylation may be a novel regulatory mechanism of PPARγ activation by MST pathway.

Introduction

➢**MST kinase pathway**

- Identified as 'Hippo pathway' in *Drosophila.*
- Mutation (loss-of function) of Hpo pathway resulted in significant overgrowth of organs.
- Activation of Hpo pathway resulted in reduction of cell proliferation and increased sensitivity to developmentally regulated apoptosis.

➢**PPARg**

- Nuclear receptor
- Master regulator in adipocyte differentiation

➢Previously, we showed that MST2 activates PPARγ and induces adipocyte differentiation.

➢In this study, we identified a novel MST2-induced Ser phosphorylation site in PPARγ and analyzed its function.

Results

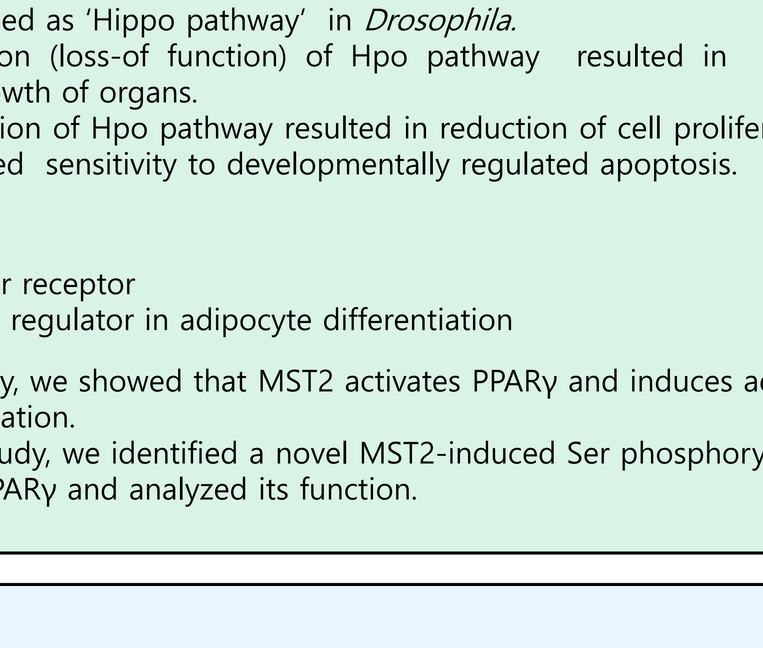


Fig 1. Identification of PPARγ phosphorylation site by Mass spectrometry

(A) HEK293 cells were transfected with Myc-PPARγ and/or MST2 and SAV1. Equal amounts of cell lysates (6 mg) were immunoprecipitated with anti-Flag antibody, separated in an SDS-PAGE and stained with Coomassie Blue. The PPARγ bands as indicated with boxes were excised and sent for MS-Spectrometric analysis. (B) LC/MS spectrum of SIVA1 phosphopeptide corresponding to the unambiguous identification of phosphorylation at Ser492 in PPARγ. (C) A schematic diagram showing the position of Ser492 in PPARγ protein.

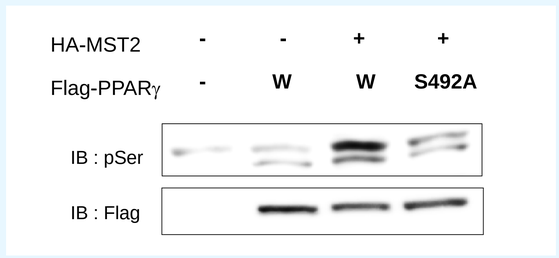


Fig.2. Serine phosphorylation of PPARγ was detected by phosphoSer492-specific antibody.

pSer492 specific antibody was prepared with a phosphopeptide containing pSer492 as an immunogen in a rabbit. The antiserum was purified with affinity chromatography. HEK-293 cells were transfected with vectors for wild type or mutant PPARγ and MST2. 48hr after transfection, cell lysates were analyzed with a rabbit pSer-specific serum.

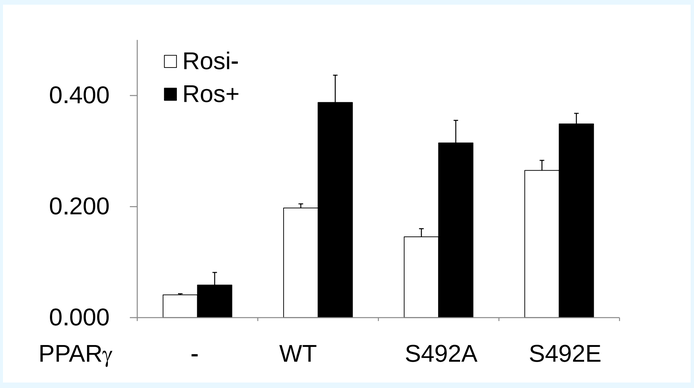


Fig. 3. Effect of Ser492 phosphorylation on the transcriptional activity of PPARg .

U2OS cells were transfected with aP2-luc reporter plasmid and vectors for PPARg of wild type or mutants. 24 hr after transfection, the cells were treated for 16 hr with (black bar) or without (white bar) 10 μM of rosiglitazone. Cell lysates were analyzed with dual-luciferase reporter assay kit. Values are expressed as the mean ± SD (n = 3).

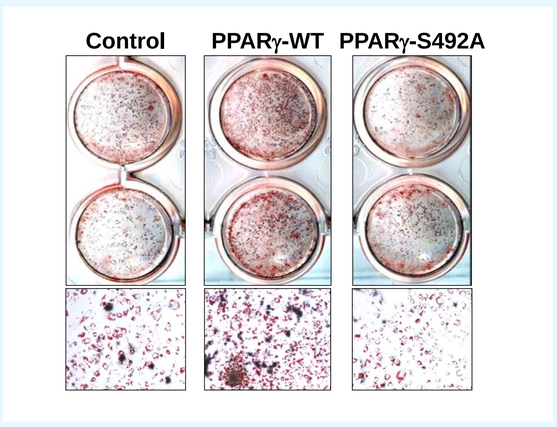


Fig. 4. Effect of Ser492 phosphorylation on adipocyte differentiation of 3T3-L1 cells induced by PPARg.

3T3-L1 preadipocytes were transfected with wild type or S492A mutant of PPARγ and then stimulated with the full differentiation cocktail. The cells were differentiated for 10 days and stained with Oil red O reagent and photographed.

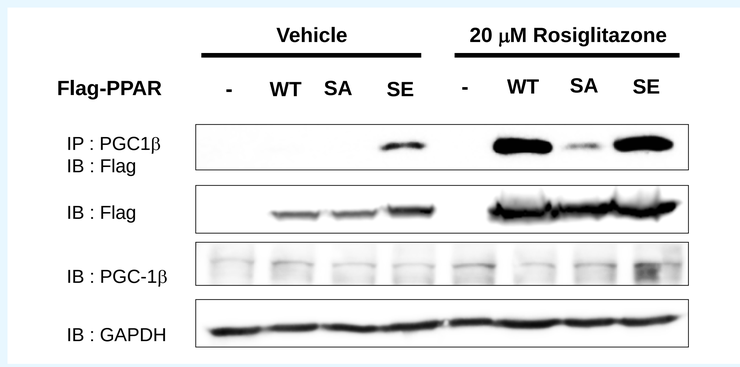
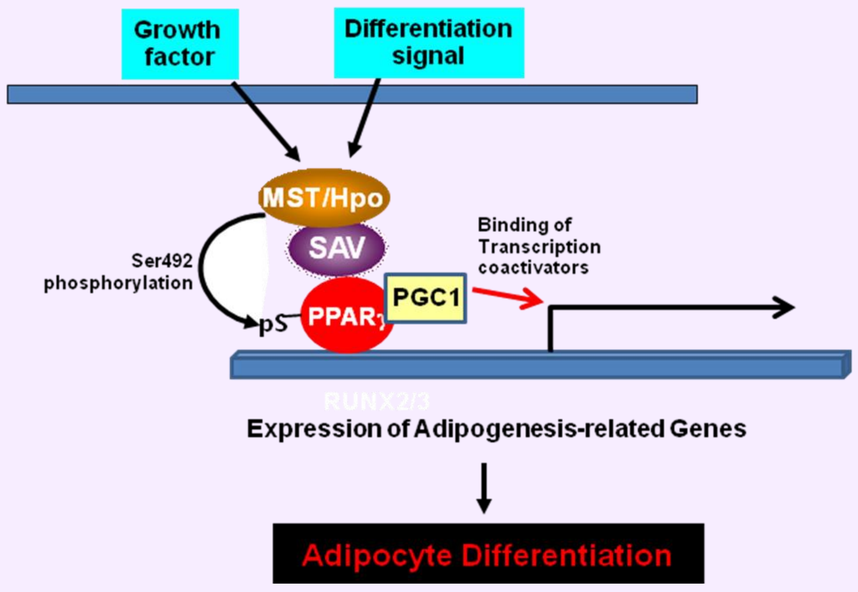


Fig. 5. Interaction of PGC1β with wild type or phospho-mutant PPARg.

HEK-293 cells were transfected with vectors for wild type or mutant PPARg . 24 hr after transfection, the cells were treated for 16 hr with or without 20 μM of rosiglitazone. Cell lysates were analyzed with immunoblot and co-immunoprecipitaion.

Conclusion



1. **MST2 phosphorylated Ser492 residue within ligand binding domain (LBD) of PPARγ.**
2. **The identified Ser492 phosphorylation was required for transactivation capability and adipogenetic activity of PPARγ .**