Supplementary information

Peroxisome Proliferator-Activated Receptor gamma as a Theragnostic Target for Mesenchymal-type Glioblastoma Patients

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The supplementary information contains 6 figures and 4 tables
Fig. S1. Analysis of PPARγ and COUP-TFI expression in PN or MES GSCs versus normal human astrocyte (NHA) or normal neural stem cell (NSC). (a) High expression of PPARγ in MES GSCs (left) or COUP-TFI in PN GSCs (right) compared to NHA and NSC analyzed by RNA-seq. (b) Prognostic value of COUP-TFI in GBM. Kaplan-Meier plots were represented for survival of GBM patients upon COUP-TFI expression in public database. Overall survival (left, n=206) and disease free survival (middle, n=162) were analyzed using TCGA dataset.
**Fig. S2.** *In vitro* cell viability assay upon multiple treatments. PN or MES GSCs were treated with 15d-PGJ2 (a), troglitazone (b) or T0070907 (c) in a dose dependent manner for 7 days and followed by MTS assay for cell viability analysis. Value are mean ± SEM (n=3). (d) Sphere forming capability upon pioglitazone treatment in GSCs. Photos of PN and MES GSCs upon 10 μM of pioglitazone treatment for 14 days. Scale bar represents 100 μm. (e) Oxygen consumption rate (OCR) in MES GSCs with pioglitazone treatment. Cells were treated with 10 μM of pioglitazone for 2 days followed by measuring OCR as described in method.
Fig. S3. Exogenous expression of PPARγ serves as a tumor suppressor in PN GSCs. (a) mRNA expression of PPARγ and target genes in PN GSCs. PN GSCs were transduced with pAd-Dest control or pAd-PPARγ overnight followed by 10 μM of pioglitazone treatment for 24 h. Data represent mean ± S.E.M. (n=3). (b) In vitro cell viability assay upon PPARγ overexpression and/or activation. PN GSCs were transduced with adenovirus harboring pAd-Dest control or pAd-PPARγ overnight followed by 10 μM of pioglitazone treatment for 3 days. Cell viability was assessed using MTS assay. Asterisks refer to ** P < 0.01, *** P < 0.001, **** P < 0.0001 (one-way ANOVA, Tukey’s post-hoc test). (c) mRNA expression of CD44 in MES
GSCs infected with the corresponding adenoviruses. Cells were transduced overnight with adenoviruses with control or PPARγ expression plasmid and followed by pioglitazone 10 μM treatment for 24 h. Data represent mean ± S.E.M. (n=3). (D) Representative pictures of morphology of PN GSCs with pAd-Dest control or pAd-PPARγ in TNFα-induced PMT process. PN cells were daily treated with 50 ng/mL of TNFα for 4 days in the presence of adenovirus expression pAd-Dest control or pAd-PPARγ. Scale bar represent 500 μm.
Fig. S4. Monitoring body weight change of the *in vivo* tumor models. (a) MES 83 GSCs were xenografted into the flank region of nude mice. Mice were intraperitoneally administered with vehicle (n=4) or pioglitazone 100 mg/kg (n=5) for 31 days. Body weights were measured every other day and relative body weights are shown as mean relative body weight ± SEM. (b) Body weight of mice intracranially injected with MES 83 GSCs with vehicle (n=5) or pioglitazone 100 mg/kg (n=5). Body weights were measured every day.
**Fig. S5.** PPARγ expression in GBM patients. (a) MRI images (upper) and immunohistochemistry for PPARγ, SOX2 and CD44 expression (lower) in primary (left) tumor and recurred tumor (right) from the same patient. (b) Immunoblot analysis for PPARγ expression in GBM or brain meningioma tissues. Scale bar: 50 μm.
Fig. S6. Pharmacological assessment for potential upstream factors regulating PPARγ expression in GBMs. (a) MES GSCs were treated with autophagy inhibitor bafilomycin A1 for 36 h followed by immunoblot for PPARγ and LC3 expressions. (b) PN and MES GSCs were treated with DNA methylation inhibitor Azacitidine for 72 h followed by immunoblot for PPARγ expression. (c) MES 1123 GSCs was treated with
C/EBPβ inhibitor helenalin in 48 h followed by immunoblot for PPARγ expression. (d) MES 83 GSCs were treated with Src inhibitor SU6656, dual Src and c-Abl inhibitor dasatinib or EGFR inhibitor gefitinib followed by immunoblot for proteins of interest. (e) Cell viability of MES GSCs upon pioglitazone (P) in combinations of multiple kinase inhibitors including Src inhibitor SU6656 (SU), dual Src and c-Abl inhibitor dasatinib (Das), EGFR inhibitor gefitinib (Gef) and MEK inhibitor U0126 (U) for 7 days. Data represent mean ± S.E.M.
Table S1. Stem cell frequency of PN or MES cells treated with pioglitazone

|          | 1/(stem cell frequency) |              |          |
|----------|-------------------------|--------------|----------|
|          | Veh                    | Pioglitazone | $P$ value|
| PN448T   | 7.92                    | 9.52         | 0.476    |
| PN X01   | 28.2                    | 29.4         | 0.819    |
| PN X02   | 25.3                    | 28.4         | 0.54     |
| MES 0502 | 4.6                     | 12.5         | 0.000373 |
| MES 1123 | 33.6                    | 93.8         | 2.35E-07 |
| MES 83   | 30.9                    | 160.5        | 7.98E-15 |
Table S2. Subtype analysis of GBM patients in the TCGA datasets.

|                  | Overall Survival | Disease Free Survival |
|------------------|------------------|-----------------------|
|                  | Lower | Middle | Upper | Lower | Middle | Upper |
| Total            | 49    | 105    | 52    | 39    | 81     | 42    |
| Censored         |       |        |       |       |        |       |
| PN               | 2     | 5      | 5     | 0     | 1      | 1     |
| CL               | 1     | 1      | 1     | 0     | 0      | 0     |
| N                | 0     | 3      | 1     | 0     | 1      | 0     |
| MES              | 0     | 0      | 3     | 0     | 0      | 1     |
| US               | 0     | 0      | 0     | 0     | 0      | 0     |
| Event number     |       |        |       |       |        |       |
| PN               | 47    | 100    | 47    | 39    | 80     | 41    |
| CL               | 22    | 27     | 4     | 18    | 22     | 5     |
| N                | 17    | 34     | 1     | 14    | 28     | 1     |
| MES              | 1     | 9      | 15    | 1     | 9      | 12    |
| US               | 3     | 29     | 21    | 3     | 20     | 20    |

PN: proneural; CL: classical; N: neural; MES: mesenchymal; US: unknown subtype
| Gene name | Sequence (5’-3’) |
|-----------|-----------------|
| 18S       | Forward: ACCGCAGCTAGGAATAATGGA  
Reverse: GCCTCAGTTCCGAAAAACCA |
| SOX2      | Forward: AACCCCAAGATGCAACAACTC  
Reverse: CGGGGCCGGTATTTTATAATC |
| OLIG2     | Forward: CTCCTCAAATCGCATCCAGA  
Reverse: AGAAAAAGGTACATCGGGCCTC |
| CD44      | Forward: TACAGCATCTCTCGGACGGA  
Reverse: CCCCCATATGAAACCACACCTTC |
| BCL2A1    | Forward: ATGGATCAAGGCAAACCCGAG  
Reverse: TGGAGTGTCCCTTTCTGGTCA |
| ALDH1A3   | Forward: TCTCGACAAAAGCCCTGAAGT  
Reverse: TATTCGGCCAAGCCTATT |
| WT1       | Forward: TACACACGCACGTTGTCTTCA  
Reverse: CTCAGATGCCGACCGTACAAG |
| PPARA     | Forward: AGATCAGTGGTGGAGGTTCA  
Reverse: GGAGATGCGAGGTCAGATT |
| MMP14     | Forward: GAGCATTCCAGTGACCCCTC  
Reverse: ACCCTGACTACCCCTATAA |
| MMP2      | Forward: GCTTCCAGGGGAATCCCTAT  
Reverse: AACAGTTGACATTGGGTC |
| FSCN2     | Forward: AGCCACACAAGTTTCTGCA  
Reverse: TGGGGGCCGGACAAAT |
| CYCLIN D1 | Forward: CGTGCGCTCTTAAGATGAAGGA  
Reverse: CCGGTGAGATGCACAGCTTC |
| IL6       | Forward: CAGTTCTGCAGAAAAAGGCAA  
Reverse: ATTTGTGGTTGGTGCAAGGG |
| COX2      | Forward: AGAAAACGTCTACAAACCGGA  
Reverse: TGGCAGTGTGTTGGAGGTGG |
| P21       | Forward: GGAGACTCTCGGAGGCTAAA  
Reverse: GGGGCGCATGCGCTTTGACAT |
| PAI1      | Forward: GCCTCGGTGTGCTGCCATGCT  
Reverse: GGGGCGCATGCGCTTTGACAT |
| FABP4     | Forward: ATGGGGGTGTCCTGGTACAT  
Reverse: GACGCATTCCACCACCAGTTT |
| LPL       | Forward: CCGCGACACAAAGGAAGAGAT  
Reverse: TAGCCACGGACTCTGCTACT |
| PDK4      | Forward: TAAAGGTCTAAGCAACTAAAGGT  
Reverse: CACACATTTCCACATTGTGAT |
| TGM2      | Forward: ATGGCAGCTCCGGGAGC  
Reverse: ATCTGTACACCATAATTCCT |
| siRNA                     | Sequence (5'-3')                                           |
|---------------------------|------------------------------------------------------------|
| PPARγ siRNA 1 Sense       | GGGCGAUCUUGACAGGAAA(dTdT)                                   |
| PPARγ siRNA 1 Antisense   | UUUCCUGUCAAGAUCCGCCC(dTdC)                                  |
| PPARγ siRNA 2 Sense       | GGAAGACAACAGACAAAU(dTdT)                                   |
| PPARγ siRNA 2 Antisense   | AUUUGUCUUGUCUUUCC(dTdG)                                    |
| PPARγ siRNA 3 Sense       | GGAUGCAAGGGUUUCUUC(dTdT)                                   |
| PPARγ siRNA 3 Antisense   | GGAAGAAACCCUUUGCAUCC(dTdT)                                 |