PPARγ2 Regulates Adipocyte Differentiation of Bone Marrow Mesenchymal Stem Cells

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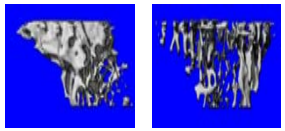
Introduction

PPARγ controls osteoblast and adipocyte development from common bone marrow mesenchymal progenitor cells. Rosiglitazone (Rosi) is a member of the thiazolidinedione class of drugs approved by the FDA to lower blood glucose levels in diabetic patients. This anti-diabetic agent can improve insulin sensitivity through the activation of the nuclear receptor PPARγ. However, PPARγ activation has been implicated in the pathophysiology of osteoporosis, obesity and other genetic disorders. *In vitro,* in the model of marrow mesenchymal cell differentiation U-33/γ2 cells (U-33 osteoblastic cells ectopically expressing PPARγ2 transcription factor) activation of the PPARγ2 isoform with rosiglitazone induces adipocyte and suppresses osteoblast phenotype, simultaneously *(Lecka-Czernik et al., J. Cell. Biochem., 1999).* As shown below, micro-CT representative renderings of proximal tibia from control and rosiglitazone-treated mice illustrate a loss of bone in treated animals (*Rzonca et al., Endocrinology, 2004).*

The effect of rosiglitazone on murine bone



control **rosiglitazone**

Activation of PPARγ by rosiglitazone suppresses components of the insulin-like growth factor regulatory system *in vitro* and *in vivo*. For instance, in a microarray experiment incorporating a full factorial design Rosi reduced transcript levels of IGF-I, IGF-II, IGFBP-4, and the type I and II IGF receptor (IGF1R and IGF2R) at 72h in U-33/γ2 compared with U-33/c cells (Lecka-Czernik et al., 2007).

A separate analysis of the same data showed that PPARγ2 regulates a molecular signature of marrow mesenchymal stem cells after 72h (Shockley et al., 2007, in press). It was found that PPARγ2 affects the expression of genes specific for the maintenance of the stem cell phenotype. Rosi and PPARγ2 combined to specifically affect "stemness" genes, including ABCG2, Egfr and CD44.

Aims

(1) Determine mechanisms by which PPARγ2 exerts its [a] anti-osteoblastic and [b] pro-adipocytic effects in mMSC

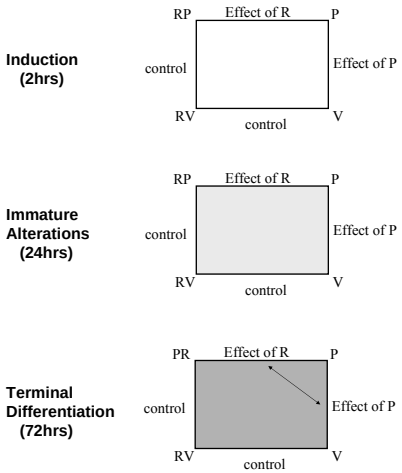
(2) Discover pro-osteoblastic networks regulated by PPARγ2

(3) Assess and take into account the effect of Rosi-independent PPARγ2 activity

Method

The antidiabetic drug rosiglitazone (R) was applied to cell cultures transfected with the pro-adipogenic peroxisome proliferator-activated receptor (PPAR)-gamma transcription factor (P, U-33/γ2) or a control vector (V, U-33/c). Thus, there were four distinct cell states corresponding to induction status (±R) of each cell type (±PPARγ). Duplicate time series experiments were performed (2-, 24- and 72-hrs after induction with R). Altogether, a total of 24 samples were placed on Affymetrix Mouse Genome 430 2.0 GeneChip® arrays for microarray analysis.

Figure 1: Experimental Design



Legend. Factorial design for the study of PPARγ-induced cell differentiation in murine mesenchymal stem cell cultures. The double-headed arrow indicates conditioning.

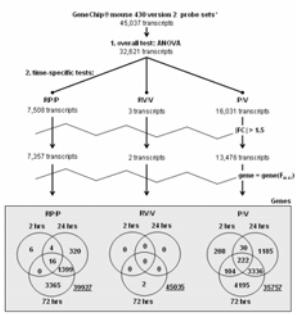
An analysis of variance (ANOVA) model was used to account for different sources of variation when conducting statistical tests. Here, Y_i represents the log-transformed gene expression measures in sample *i*.

$$Y_i = \mu + \text{CONDITION} + \varepsilon_i$$

This model accounts for differences in expression due to the average signal for a gene (μ), the twelve levels of treatment condition (**CONDITION**), and random error (ε_i). Focused research hypotheses were tested using a similar modeling approach after subsetting the data into the desired pairwise comparison.

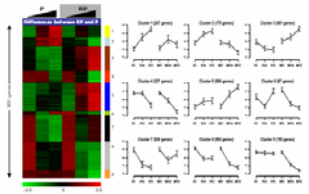
Results

Figure 2: Summary of Statistical Tests

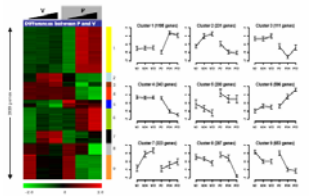


Legend. An overall F-test was used to resolve differences between experimental states (i.e., any cell state at a given harvest time). Pairwise contrasts were used to find significant differences due to activation of the U-33/γ2 cell type (RP/P), differences between activated and inactivated control cells (RV/V), or differences between cell type (P/V). The resulting lists of differentially expressed transcripts (DETs) were filtered by a 1.5-fold change. When multiple DETs were mapped to a single Entrez gene, the DET with the largest Fs statistic was chosen to represent the gene.

Figure 3: Expression Patterns



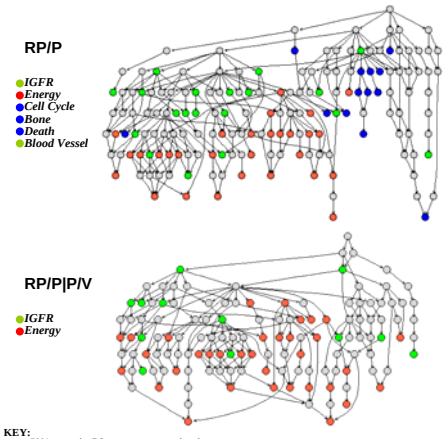
Process	Cluster(s)
Signaling	*2,3,6,*7,**8,**9
Immune	**1,2,**4,**5,***6*
Energy	*2,***3,5,**7
Bone	**1**
Death	**1,7,***8*
Cell Cycle	**4,5,8**



Process	Cluster(s)
Signaling	**1,3,***5,***7**
Immune	**1,***4,***6,***7,8**
Energy	**2,4,6,9**
Bone	*1,7*
Death	*9*
Cell Cycle	**2,***3,4,7,***8,***9*

Legend. K-means clustering of log2 transformed expression estimates standardized across experimental states. Time (2, 24 or 72 h) is indicated with black triangles above each heat plot. Association of biological processes containing at least two genes with clusters are shown in bold for highly significant ($p<0.01$) and stable ($p<0.05$) thresholds.

Figure 4: GO DAG of Terminal Differentiation



KEY:
- >70% genes in GO term are up-regulated
- >70% genes in GO term are down-regulated
- ≤ 70% of genes in GO term are regulated in one direction

Legend. DETs for RP/P were mapped to Entrez genes and tested for association with GO biological process terms. Summary descriptions of important processes in each DAG (e.g., IGFR) are indicated under graph descriptors along with colored circles indicating direction of change.

Conclusion

- ANOVA can be used to find time-dependent expression differences within and between murine cell types
- PPARγ2 is a major regulator of mMSC differentiation
- Rosiglitazone activation of PPARγ2 transfected cells results in hierarchical interactions between different regulatory pathways
- PPARγ2 has a pleiotropic effect on the expression of certain biological processes, including IGFR signaling and angiogenesis

Useful References

Lecka-Czernik B, Ackert-Bicknell C, Adamo ML, Marmolejos V, Churchill GA, Shockley KR, Reid IR, Grey A, Rosen CJ (2007) Activation of peroxisome proliferator-activated receptor γ (PPARγ) by rosiglitazone suppresses components of the insulin-like growth factor regulatory system *in vitro* and *in vivo*. Endocrinology 148, 903-911.

Shockley KR, Rosen C, Churchill GA, Lecka-Czernik B (2007) PPARγ2 regulates a molecular signature of marrow mesenchymal stem cells. PPAR Research (in press).