EPDR1 Governs Metabolic and Immunologic Re-programming of Human Mesenchymal Stem Cells during Osteoblast Differentiation

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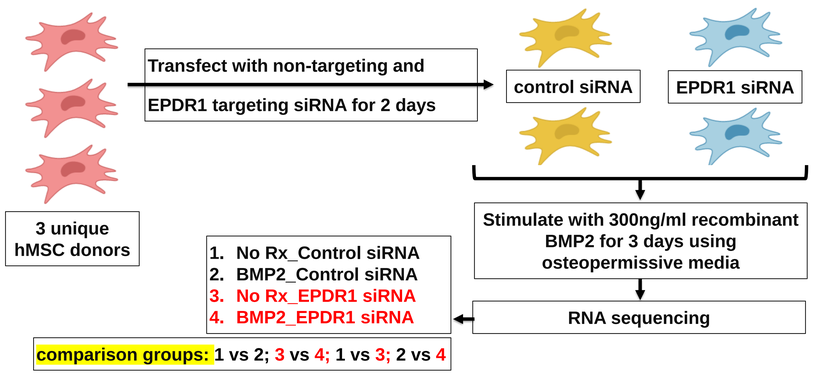
Introduction

- ***EPDR1*** **is a novel human osteoblast regulatory gene previously identified by intersecting BMD GWAS datasets with promoter-focused Capture-C and ATAC-seq generated in differentiating human osteoblasts[1].**
- **The open chromatin region harboring the BMD variants rs1524068, rs6975644 and rs940347 functions as an osteoblast specific *EPDR1* enhancer in differentiating cells[2].**
- **EPDR1 knock-down in human mesenchymal stem/progenitor cells (hMSC) diminishes osteoblast differentiation but favors adipogenic differentiation[1].**
- **Crystal-structure of EPDR1 protein suggests human EPDR1 folds into a dimer using a monomeric subunit consisting of a deep hydrophobic pocket to bind to hydrophobic fatty acids and function as a lipoprotein carrier[3]. However, the precise molecular processes affected by EPDR1 silencing of differentiating human osteoblasts remain unknown.**

Objective

To determine molecular processes regulated by *EPDR1* during human osteoblast differentiation through transcriptomic analyses

Methods



Results

1. The total number of differentially expresses genes varies among different comparisons

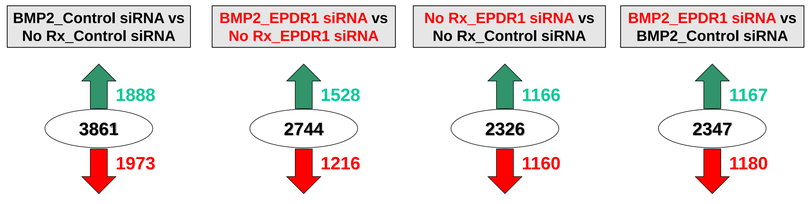


Figure 1: Total number of differentially expressed genes compared across siRNA and BMP2 stimulated conditions is depicted.

2. EPDR1 mRNA and protein levels are decreased by EPDR1 siRNA transfection of hMSC

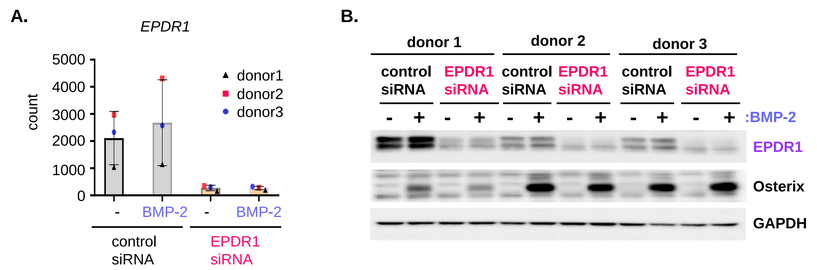


Figure 2: (A) Normalized *EPDR1* counts in three donors across transfection and BMP2 stimulation is shown. **(B)** Immunoblot analysis of the total cell lysate prepared from parallel samples used for RNA sequencing also depicts EPDR1 knock-down. Osterix expression was determined to show BMP/SMAD signal transduction in the transfected cells. GAPDH was examined as an internal control.

3. Non-targeting control siRNA transfected cells show regulation of BMP signaling and skeletal developmental GO processes in the presence of BMP

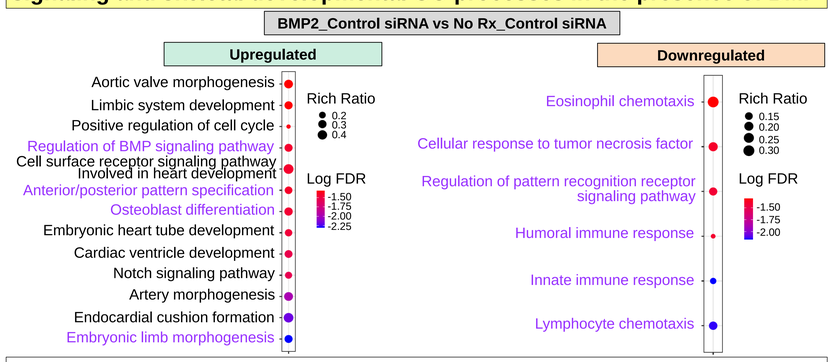


Figure 3: Upregulated (Left panel) and Downregulated (Right panel) molecular processes modulated by BMP in non-targeting control siRNA transfected cells is shown. Upregulated processes show relevance for skeletal development (purple), cardiovascular development and Notch signaling. All of the downregulated molecular processes are relevant for immune responses (purple).

DEGs with FDR <0.05 did not show any significant GO processes between BMP2_EPDR1siRNA vs No Rx_EPDR1 siRNA

4. Immunologic and metabolic processes are differentially upregulated in EPDR1 silenced cells whereas cell division is downregulated

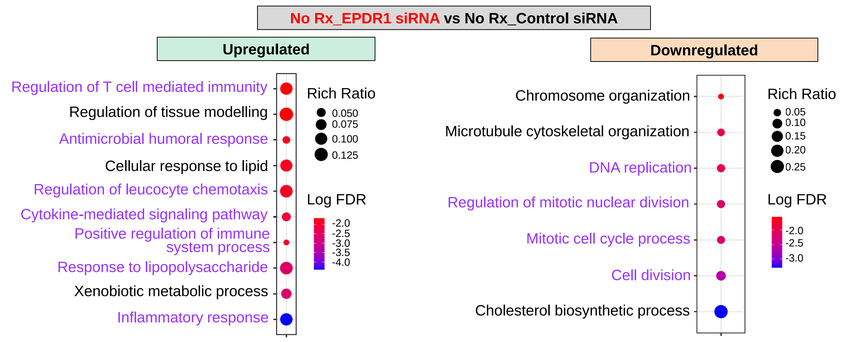


Figure 4: Top 10 upregulated (Left panel) and Downregulated (Right panel) molecular processes in EPDR1 siRNA compared to non-targeting control siRNA transfected cells is shown. Upregulated processes show relevance for immune responses (purple), responses to lipid and xenobiotic metabolism. Majority of the downregulated molecular processes are related to cell division (purple).

5. Immunologic profile of EPDR1 silenced cells are maintained under BMP2 stimulated conditions

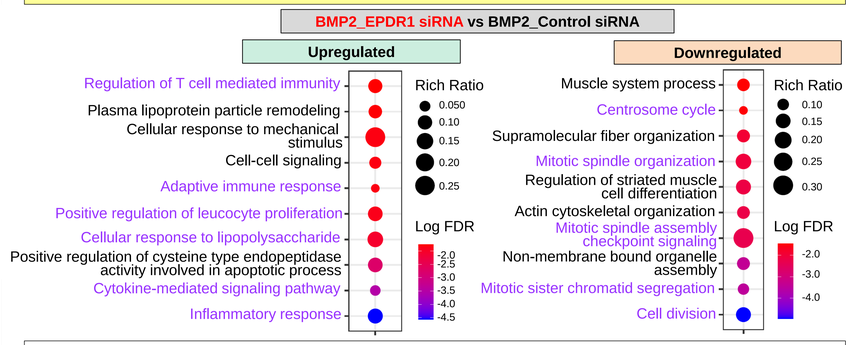


Figure 5: Top 10 upregulated (Left panel) and top 10 Downregulated (Right panel) molecular processes in EPDR1 siRNA compared to non-targeting control siRNA transfected cells in BMP2 stimulated condition is shown.

6. Additional molecular processes upregulated by EPDR1 silencing in differentiating osteoblasts further supports inflammation, bone catabolism and fatty acid metabolism

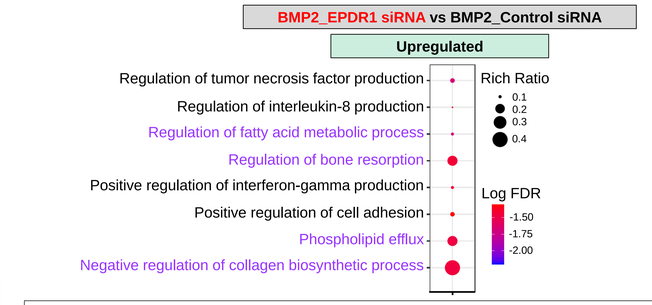
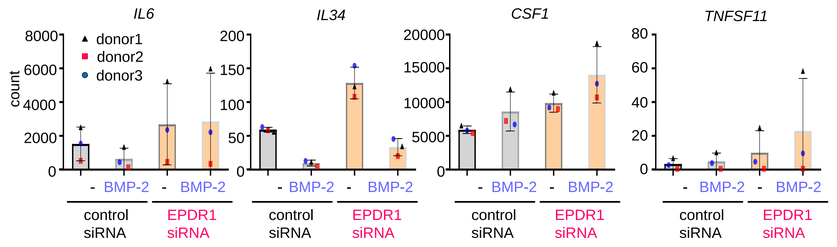


Figure 6: Additional molecular processes upregulated in EPDR1 silenced human osteoblasts that are relevant for bone catabolism and lipid metabolism (purple) are shown.

6. Examples of selected DEGs associated with bone catabolism



Summary and Discussion

- ***EPDR1*** **silencing in differentiating human osteoblasts results in a general decrease in molecular processes required for osteoblast differentiation and skeletal development**
- **Loss of *EPDR1* in progenitor cells increases immune response and lipid metabolism related molecular processes but decreases cell division. All these molecular processes remain analogous during hMSC osteoblastic differentiation**
- ***EPDR1*** **silencing enhances expression of cytokine(s) and bone catabolism related genes in differentiating human osteoblasts**
- **Collectively, these results suggests EPDR1 function to maintain cellular homeostasis and may affect both bone anabolism and catabolism *in vivo.***

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References: 1. Chesi A, Wagley Y et. al., *Nat Commun. 10 (1):1260* (2019); **2.** Pippin JA, Chesi A, Wagley Y et. al., *JBMR Plus 5(9):e10531* (2021); **3.** Wei Y, Xiong ZJ, Chunxia Z et. al., *Commun. Biol. 2:52* (2019).