Novel Human Osteoblast Effector Gene *EPDR1* Demonstrates Differing Outcomes after Knock-down or Knock-out in Murine *In vitro* Osteoblastogenesis and During Murine Bone Modeling

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Introduction

- ***EPDR1*** **is a recently characterized human osteoblast effector gene previously identified by intersecting BMD GWAS data with functional genomics in differentiating human mesenchymal stem/progenitor cells (MSC)[1].**
- **The open chromatin region harboring BMD-associated variants rs1524068, rs6975644 and rs940347 functions as an osteoblast specific *EPDR1* enhancer in differentiating human osteoblasts[2].**
- **Loss of EPDR1 function affects metabolic and immunologic re-programming of hMSC (poster SUN-278) and favors adipogenic differentiation in lieu of osteogenic differentiation[1].**
- **Although *epdr1* has been characterized to play a role in brown fat commitment and thermogenesis via an auto- or paracrine circuit in mice[3], its role in murine MSC and bone development remains largely unknown.**

Objective

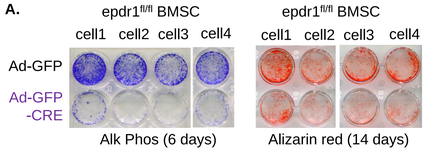
To investigate the role of *epdr1* in osteoblastic and adipogenic differentiation of mouse MSC and to dissect the effect of global loss of Epdr1 function during bone development using *epdr1* knock-out mouse model

Methods

- **Bone-marrow stromal cells (BMSC) were harvested from 1-, 3- or 6-months *epdr1*$^{fl/fl}$ and *epdr1*$^{-/-}$ mice from tibiae and femurs. Cells were propagated for 7-10 days, collected by mild trypsinization and used for osteogenic and adipogenic differentiation assays.**
- ***In vitro*** **recombination of *epdr1*$^{fl/fl}$ BMSC was accomplished using adeno-GFP-CRE infection at a MOI of 250 for 2 days before using for differentiation assays. Adeno-GFP was used as control.**
- **Both femurs from 1-, 3- or 6-months from *epdr1*$^{fl/fl}$ and *epdr1*$^{-/-}$ were harvested after double calcein injection. Left femurs were fresh frozen using PBS-soaked gauge and used for microCT reconstruction. Right femurs were fixed in 4% paraformaldehyde. Gene expression analysis was performed in total RNA harvested from tibial shaft and tibial metaphysis using standard procedure.**

Results

1. *In vitro* recombination of *epdr1*$^{fl/fl}$ in mMSC reduces osteoblastic differentiation



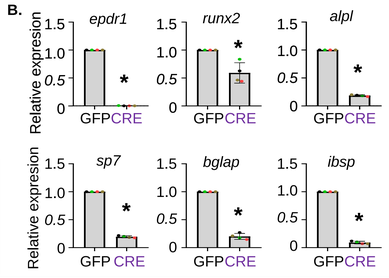


Figure 1: A. Alkaline phosphatase expression (purple, 6 days) and Alizarin red S staining (red, 14 days) was histochemically determined in four separate *epdr1*$^{fl/fl}$ MSC preparations after Ad-GFP or Ad-GFP-CRE expression. **B.** *epdr1* and selected osteoblastic gene expression after 6 days in osteopermissive media was determined.

2. *In vitro* recombination of *epdr1*$^{fl/fl}$ in mMSC reduces adipogenic differentiation accompanied by a modest reduction in *pparg* expression

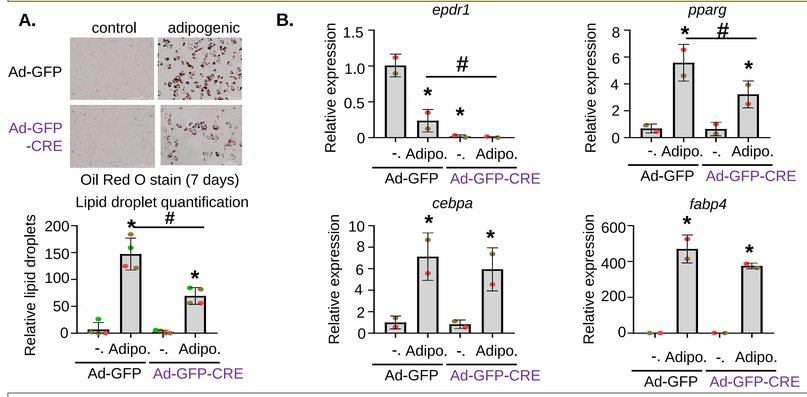


Figure 2: (A) Adipogenic differentiation was performed in four separate *epdr1*$^{fl/fl}$ BMSC preparations after Ad-GFP or Ad-GFP-CRE expression. A representative Oil Red O-stained photomicrograph is shown. Bottom histogram: Enumerated data from 4 different cell preparations is presented. **(B)** Expression of *epdr1* and adipogenic marker genes *pparg, cebpa,* and *fabp4* was determined from two different cell preparations. * P<0.05 versus no adipogenesis, #P<0.05 versus Ad-GFP Adipo group.

3. BMSC from 1 month old *epdr1*$^{-/-}$ mice show mineralization defect but not from cells harvested from 3- or 6-months old mice

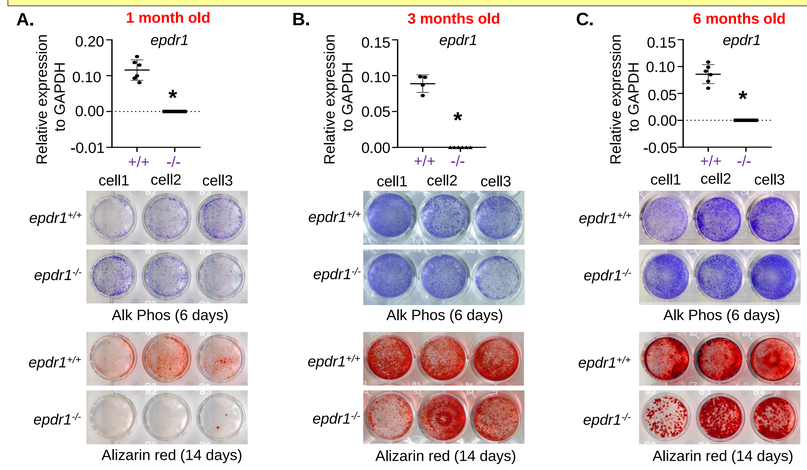


Figure 3: (A) Top: *epdr1* expression was determined in BMSC harvested from 1 month old *epdr1*$^{fl/fl}$ (+/+) and *epdr1*$^{-/-}$ mice after 6 days of culture in osteopermissive media. Alkaline phosphatase (middle panel, purple) and extracellular calcium deposition (bottom panel, red) was histochemically determined at indicated times. **(B-C)** Similar experiments were performed using cells harvested from 3 months **(B)** and 6 months old **(C)** mice. n= 4-6, *P<0.05 versus *epdr1*$^{fl/fl}$

4. Body weight and fat depot are reduced in six months old *epdr1*$^{-/-}$ *male mice.*

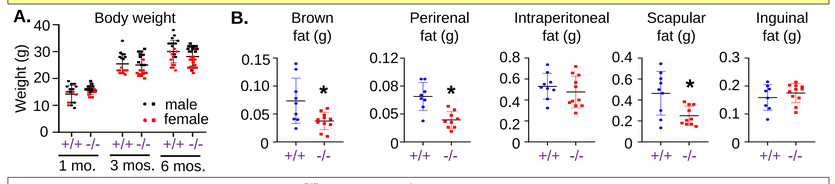


Figure 4: (A) Body weight of *epdr1*$^{fl/fl}$ and *epdr1*$^{-/-}$ mice at 1,-3-, and 6-months of age is shown). **(B)** Various fat depots were also measured in 6 months old male *epdr*$^{fl/fl}$ and *epdr1*$^{-/-}$ mice (n = 9-11).

5. Cortical and Trabecular bone properties are comparable between 6 months old *epdr1*$^{fl/fl}$ and *epdr1*$^{-/-}$ mice

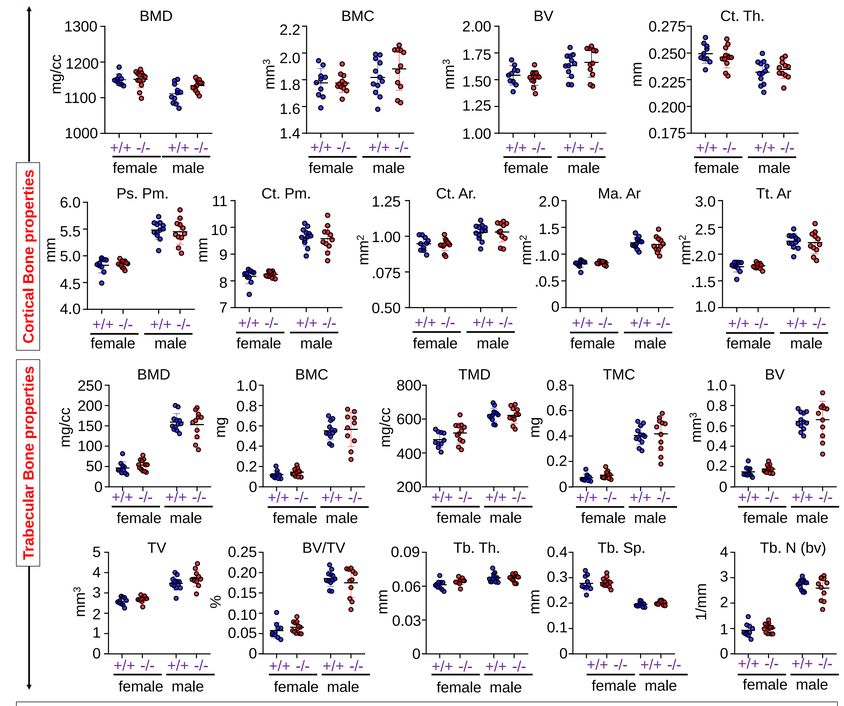


Figure 5: Cortical (top) and Trabecular (bottom) bone parameters from 6 months old mice (n = 10-13)

6. Bone metabolism associated genes in tibial metaphysis and shaft are analogous in 6 months old *epdr1*$^{fl/fl}$ and *epdr1*$^{-/-}$ mice

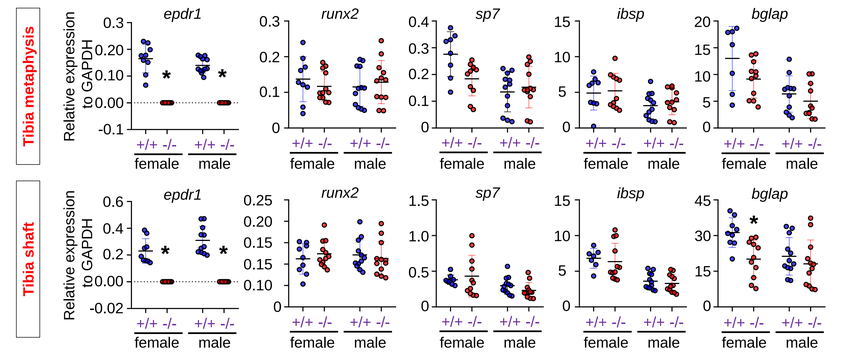


Figure 6: *epdr1* and selected genes associated with bone parameters were determined from tibial metaphysis (top panels) and tibial shaft (bottom panel) of 6 months old mice (n=7-12 for each group).

Summary

- ***In vitro epdr1*** **knock-down in murine MSC reduces osteoblastic and adipogenic differentiation**
- **Except for mineralization defect in cells from 1 month old mice and a lower body weight of 6 months old male mice, cortical and trabecular bone properties and bone relevant genes are expressed comparably in *epdr1*$^{fl/fl}$ and *epdr1* knock-out mice**
- **Future studies using conditional loss of *epdr1* are warranted to understand osteoblast and adipocyte cell lineage specific roles of *epdr1 in vivo***

Orthopaedic Research Laboratories

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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.** Deshmukh AS, Peijs L, BeaudryJL et. al., *Cell Metab. 30(5):963-975.e7.* (2019).