ERK1/2 signaling is a modulator of bone marrow mesenchymal stem cells osteogenic differentiation in high-turnover renal osteodystrophy

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Backgroud and Aim

- The mechanisms of high-turnover renal osteodystrophy are largely unknown. The bone marrow-derived mesenchymal stem cells (BM-MSC) are the foundation of bone turnover.
- The aim of this study was to investigate the BM-MSC osteogenic differentiation characteristics in uremic rat with phosphate-induced high-turnover renal osteodystrophy and their mechanisms.

Methods

- Model of uremia with high-turnover renal osteodystrophy was induced in 5/6 nephrectomy rat with high dietary phosphorus for 24 weeks, and BM-MSC was isolated from femur and tibia bone. BM-MSC from normal rat served as the control group.
- The cultured cells were identified as CD90+/CD34-/CD44+/CD45- and differentiated to osteoblast in medium containing glycerin phosphate and dexamethasone.

Results

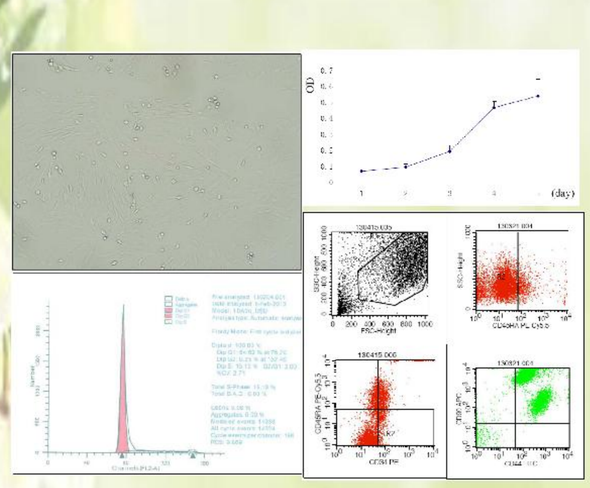
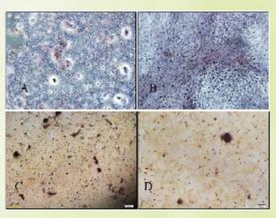
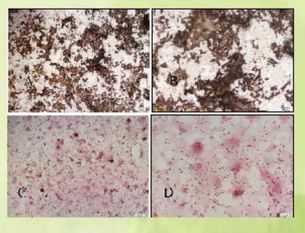


Figure 1. BM-MSC identified as CD90+/CD34-/CD44+/CD45-



A,B：ALP；C,D：OPN；IHC，

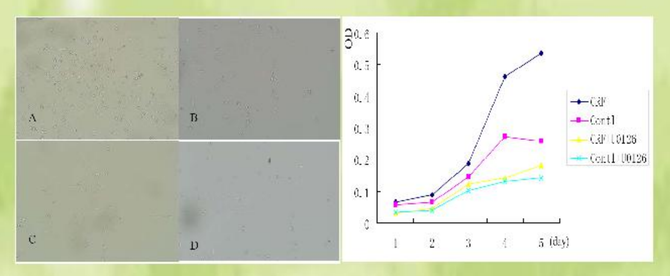
A,C×40；B,D×100



A,B：von Kossa；C,D：HE；

A,B×40；C,D×100

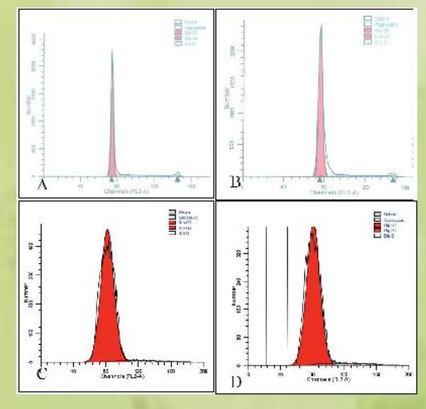
Figure 2. BM-MSC differentiated to osteoblast in medium containing glycerin phosphate and dexamethasone

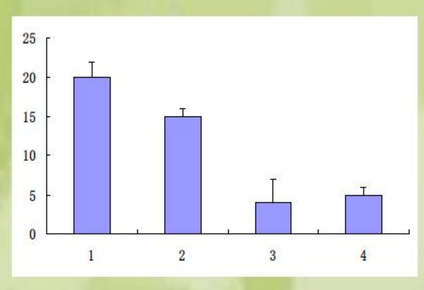


A,B,C,D : CRF, Contl, CRF+U0126, Contl+U0126 ×100

Figure 3. U0126, an ERK1/2 inhibitor, suppressed CRF BM-MSC proliferation at day 5

- The ratio of p-ERK1/2 to total ERK1/2 in the differentiated osteoblast increased significantly at the 72 h and maintained for 24 h, which was decreased significantly when added to U0126, an ERK1/2 signaling blocker.
- Compared with the control group cells, the expressions of ALP and OPN increased dramatically at day 14, and the calcium phosphate deposits in the matrix increased more obviously and the amounts of bone nodules were larger in high-turnover group cells at day 21.
- However, in the ERK1/2 blocked high-turnover group cells, ALP and OPN expressions decreased significantly at day 14 compared with the non-ERK1/2 blocked high-turnover group cells, with a weaker calcium phosphate deposits and a smaller number of bone nodules at day 21.

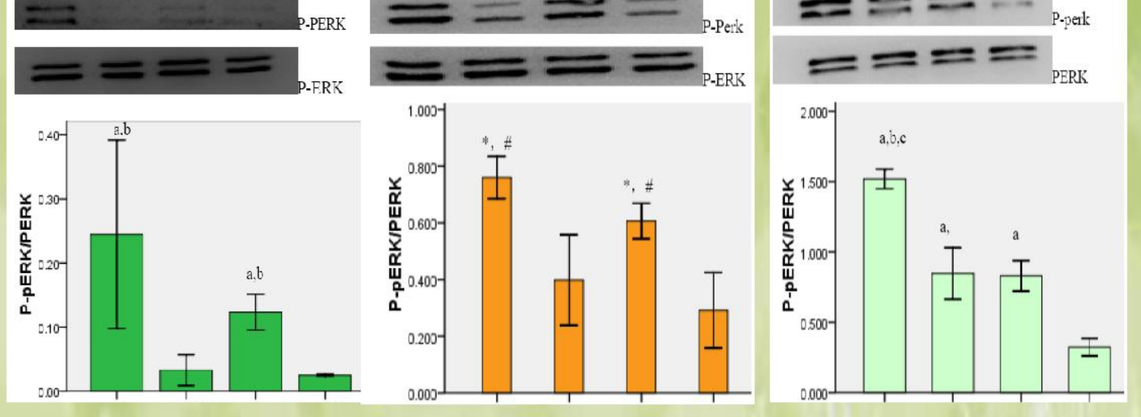




CRF Contl CRF+U0126 Contl+U0126

A,B,C,D : CRF, Contl, CRF+U0126, Contl+U0126

Figure 4. U0126, an ERK1/2 inhibitor, suppressed CRF BM-MSC proliferation at day 5



CRF,, CRF+U0126, Contl, Contl+U0126 48h

CRF, CRF+U0126, Contl, Contl+U0126 72h

CRF, CRF+U0126, Contl, Contl+U0126 96h

Figure 6. U0126 suppressed BM-MSC PERK1/2 phosphorylation at day 2, 3, 4

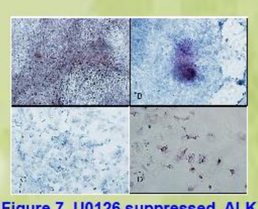


Figure 7. U0126 suppressed ALK expression in BM-MSC at day 7 （A,B,C,D : CRF, Contl, CRF+U0126, Contl+U0126; IHC, ×100 ）

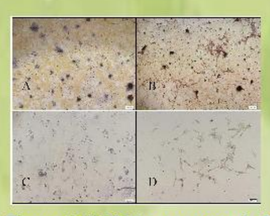


Figure 8. U0126 suppressed OPN expression in BM-MSC at day 14 （A,B,C,D : CRF, Contl, CRF+U0126, Contl+U0126; IHC×40 ）

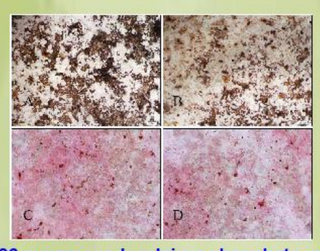


Figure 9. U0126 suppressed calcium phosphate production in BM-MSC at day 14 （A,B,C,D : CRF, Contl, CRF+U0126, Contl+U0126; von Kossa×40 ）

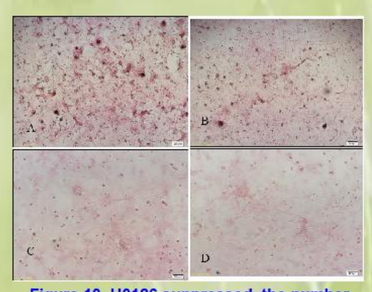
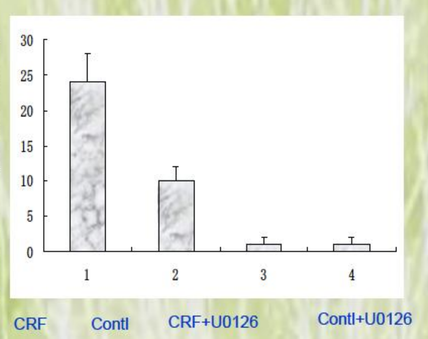


Figure 10. U0126 suppressed the number of calcium nodules in BM-MSC at day 14 （A,B,C,D : CRF, Contl, CRF+U0126, Contl+U0126; HE×40 ）



Conclusion

- Our findings provide direct evidences of the pivotal role of ERK1/2 signaling pathway in the increased osteogenic differentiation abilities of BM-MSC in high-turnover renal osteodystrophy.

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