P2-3 Osteopontin induces osteogenic differentiation by human periodontal ligament cells via calcium binding domain-ALK-1 interaction

Chaikiawkeaw D[1], Khorattanakulchai N[2], Nammultriputtar K[1], Rattanapisit K[2], Everts V[1,4], Kubera A[3], Phoolchareon W[2], Pavasant P[1].

[1]Center of Excellence in Regenerative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand.
[2]Research Unit for Plant-produced Pharmaceuticals, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand
[3]Department of Genetics, Faculty of Science, Kasertsart University, Bangkok, Thailand
[4]Department of Oral Cell Biology, Academic Center of Dentistry Amsterdam, University of Amsterdam and Vrije Universiteit, Amsterdam, The Netherlands

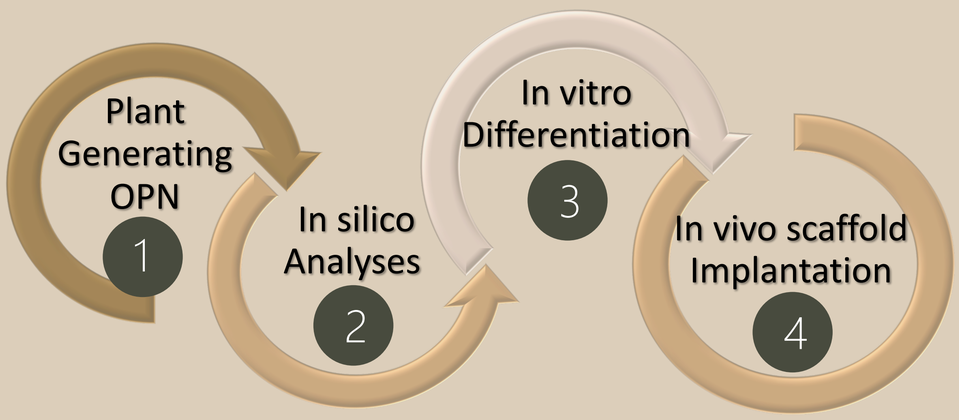
Introduction

Recently we have generated recombinant human osteopontin (rhOPN) using a plant platform (*Nicotiana benthamiana*) and demonstrated, when coated on culture plates, its osteogenic induction capacity of human periodontal ligament (PDL) cells. The aim of this study is to elucidate the molecular mechanism underlying the rhOPN-induced osteogenic differentiation of human PDL cells.

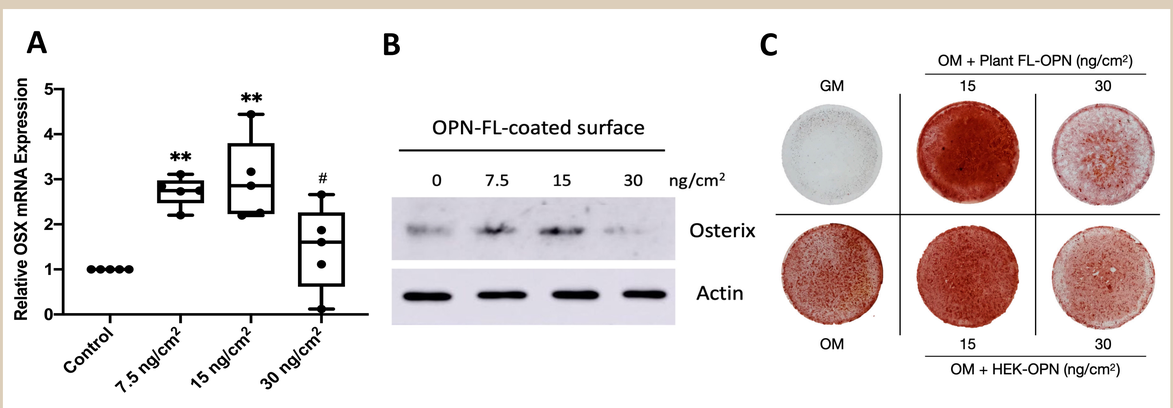
Main Finding

- **Plant generated-calcium binding domain of OPN (CaBD) induced osteogenic differentiation and new bone formation.**
- **The induction occurred via the interaction of ALK-1 receptor on PDL cells surface.**

Methods and Results

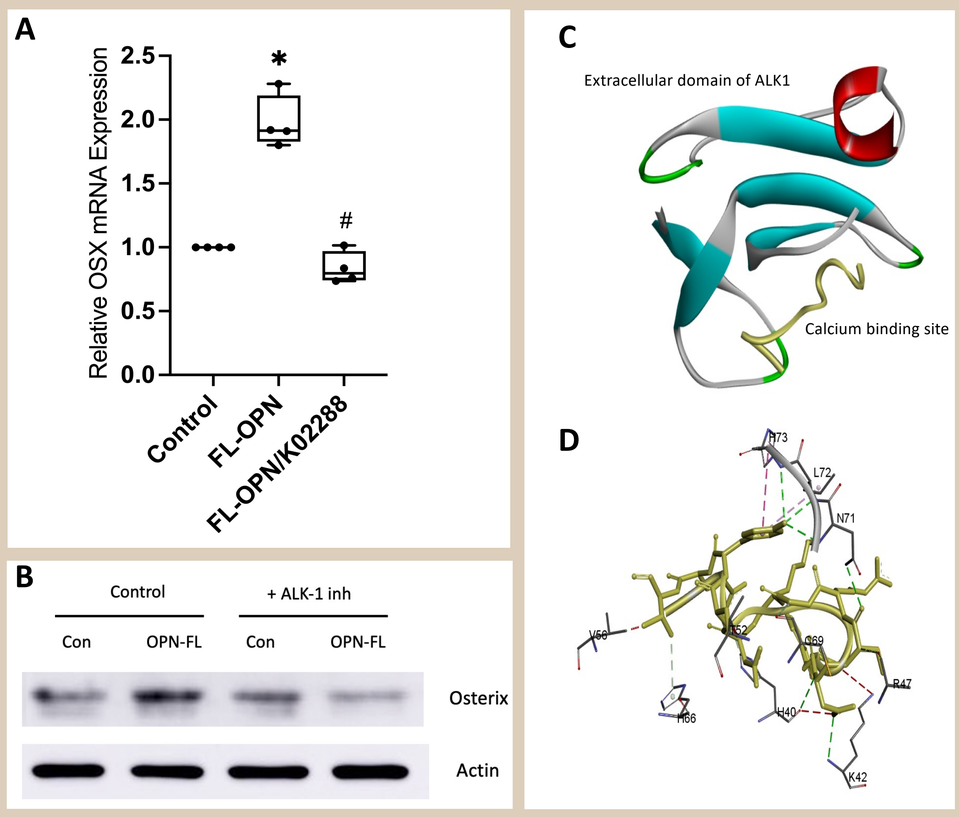


1 Fig. 1 Plant produced osteopontin stimulates osteogenic differentiation by PDL cells.



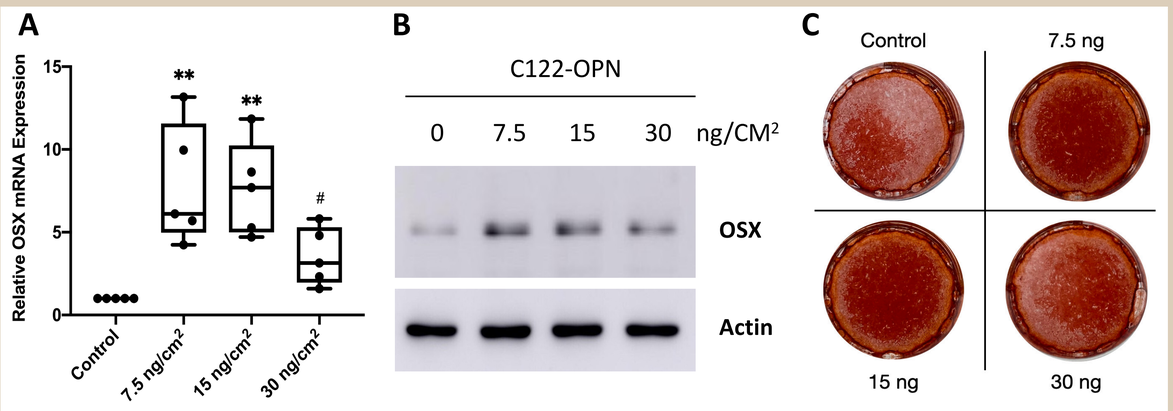
Culture plates were coated with 0-30 ng/cm^2 of plant produced-full length OPN (FL-OPN). Induction of *OSX* mRNA **(A)** and protein **(B)** could be detected in a dose-dependent manner. Increase of in vitro calcification was also detected by Alizarin Red S staining compared to HEK cells produced-OPN **(C)**.

2 Fig. 2 OPN stimulated *OSX* expression was abolished by inhibiting ALK-1 and Molecular docking of extracellular domain of ALK-1 and calcium binding domain of OPN



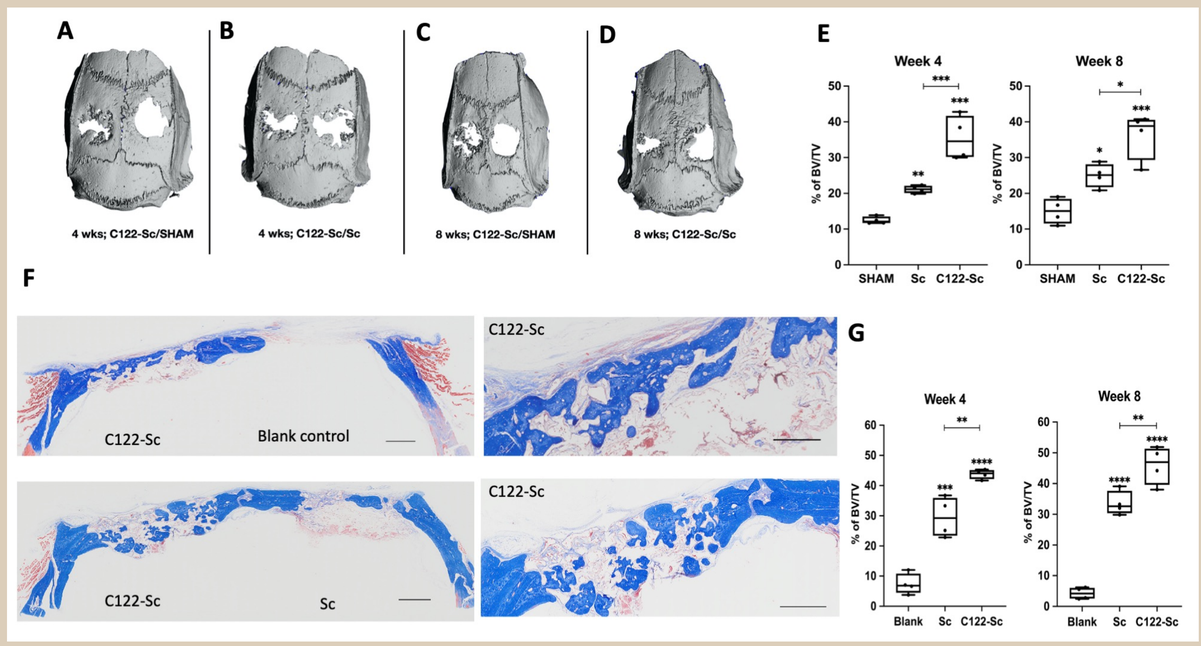
Addition of K02288, an ALK-1 inhibitor, abolished the OPN-induced expression of *OSX* as shown by RT-PCR and Western blot analysis **(A-B)**. **C)** The in silico binding of the calcium-binding domain (CaBD) (yellow) and extracellular domain of ALK-1. **D)** Interactions between the CaBD (yellow) and the extracellular domain of ALK-1. The result suggested that CaBD could bind to ALK-1 receptor and ALK-1 might be another OPN receptor that function to support osteogenesis.

3 Fig. 3 OPN calcium binding domain induced the expression of *OSX* and enhanced in vitro calcification.



To prove the function of CaBD-OPN and ALK-1, the truncated OPN contain only CaBD was generated, designated as C-122. The results from RT-PCR and Western blot analysis showed that C-122 could induce *OSX* mRNA **(A)** and protein expression **(B)**, respectively. Increase of in vitro calcification was judged by Alizarin Red S staining **(C)**, when PDL cells were seeded on C-122-coated surfaces.

4 Fig. 4 C122-coated PCL scaffold supported new bone formation in rat calvaria model.



Two circular defects with a 5-mm diameter were created in calvariae of male Wistar rats. The uncoated scaffold (Sc) or C122-coated scaffolds (C122-Sc) were embedded into the defect. The new bone formation was found in both µCT **(A-E)** and histomorphometric analysis stained with Masson's trichrome **(F-G)** at both 4 and 8 weeks.

Statistical Analysis

The data was presented as mean ± SD, statistical analyses were performed by using one-way ANOVA. *, **, *** and **** indicate significant differences from control, # indicate significant differences from 7.5 ng/cm^2. $p < 0.05$, 0.01, 0.001 and 0.0001, respectively.

Acknowledgements

This work was supported by the Chulalongkorn Academic Advancement into its second Century Project. DC was supported by The Second Century Fund, Chulalongkorn University (C2F). and PP was supported by Thailand Research Fund (RTA6180001).

Conclusion

The results suggest that next to full length OPN, the calcium binding domain of OPN, induces osteogenic differentiation and new bone formation via interaction with ALK-1 receptor.