原文題目(出處)：	Bone morphogenetic protein-2 gene controls tooth root development in coordination with formation of the Periodontium. Int J Oral Sci 2013;5:75-84
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內文：

INTRODUCTION

1. Dental follicle progenitor cells are thought to form the cementum, alveolar bone and Sharpey's fibers of the periodontal ligament (PDL). Bone morphogenetic protein-2 (Bmp2), has been shown to promote the differentiation of immortalized dental follicle cells toward an osteoblast/cementoblast phenotype.
2. Bmp2 plays a critical role in postnatal tooth development and cytodifferentiation when deleted in mature odontoblasts.
3. The role of Bmp2 in exerting its effects on the development of tooth-root, and its supportive tissues such as the periodontal ligament (PDL) and cementumas well as on pulp vasculogenesis in vivo has not been tested.
4. We investigated the role of bone morphogenetic protein-2 (Bmp2) in these processes by the conditional removal of the Bmp2 gene using the Sp7-Cre-EGFP mouse model.

MATERIALS AND METHODS

1. Sp7-Cre-EGFP mice were crossed with Rosa-LSL-tdTomato reporter mice, cryostat sections on a tape system were then visualized with confocal microscopy.
2. Bmp2 fx/fx mice were crossed with Sp7-Cre-EGFP Bmp2fx/+ to obtain wild-type (WT), heterozygotes (Het, Sp7-Cre-EGFP;Bmp2fx/1), and Bmp2-cKO$^{Sp7\text{-}Cre\text{-}EGFP}$ (Sp7-Cre-EGFP;Bmp2fx/Bmp2fx).
3. Histology, histomorphometric evaluation and acid etching of plastic embedded mandibles: Mandible tissues were collected from Bmp2-cKOSp7-Cre-EGFP
   →the left side were prepared for radiology study, and the right mandibles were prepared for bone and teeth histological evaluation.
4. Radiography: Male mice aged at 2 weeks, 1 month and 3 months were used and mandible samples were processed and stored in 70% ethanol for high-resolution X-rays.
5. Micro computed tomography analysis: Total volume of dentin, radicular (root) dentin, enamel and total pulp volume, as well as periodontal volume, was quantitated in the first and second molars in 1 month, 2 months and 3 months of age.
6. In situ hybridization
7. Immunohistochemistry
8. Immunofluorescence

RESULTS:

1.Characterization of the Sp7-Cre-EGFP model and expression in the mandible

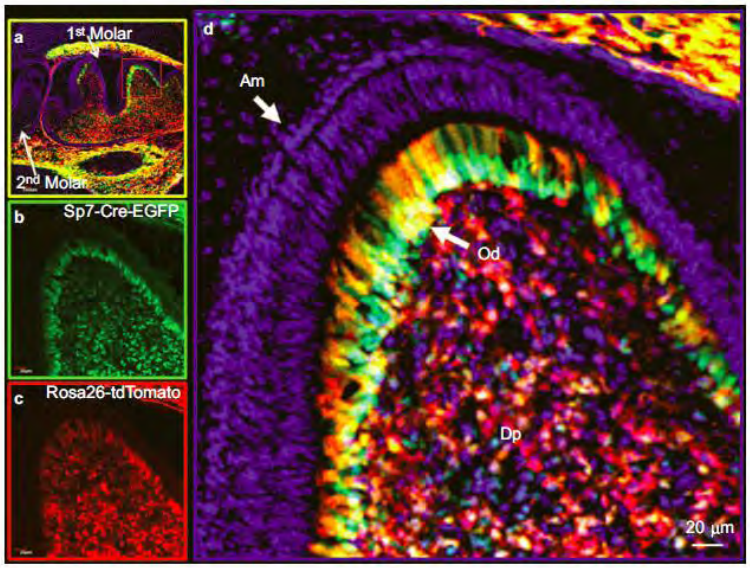


Figure 1 Lineage studies by mapping Cre activity in the first molar of a postnatal day 0 mouse by confocal microscopy using the Olympus FV 1000. Combined DAPI-stained nuclei (blue), Sp7-Cre-EGFP$^+$ (green) and tdTomato$^+$ (red) (Cre event) cells are shown. (**a**) Low magnification of the first and second molars with strong Cre activity in the alveolar bone and the first molar, but no detectable Cre activity in the second molar. (**b**) Sp7$^+$ green signal from EGFP. (**c**) tdTomato signal representing Cre events and cells derived from Cre events (red box). (**d**) Shown at high magnification (×400) of the first molar crown region. Am, ameloblasts; DAPI, 4′,6-diamidino-2-phenylindole; DP, dental-pulp chamber; Od, odontoblasts.

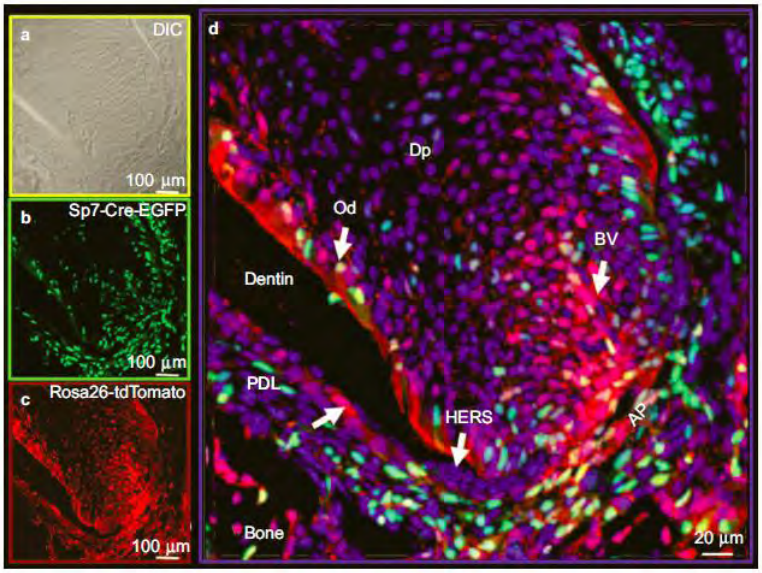
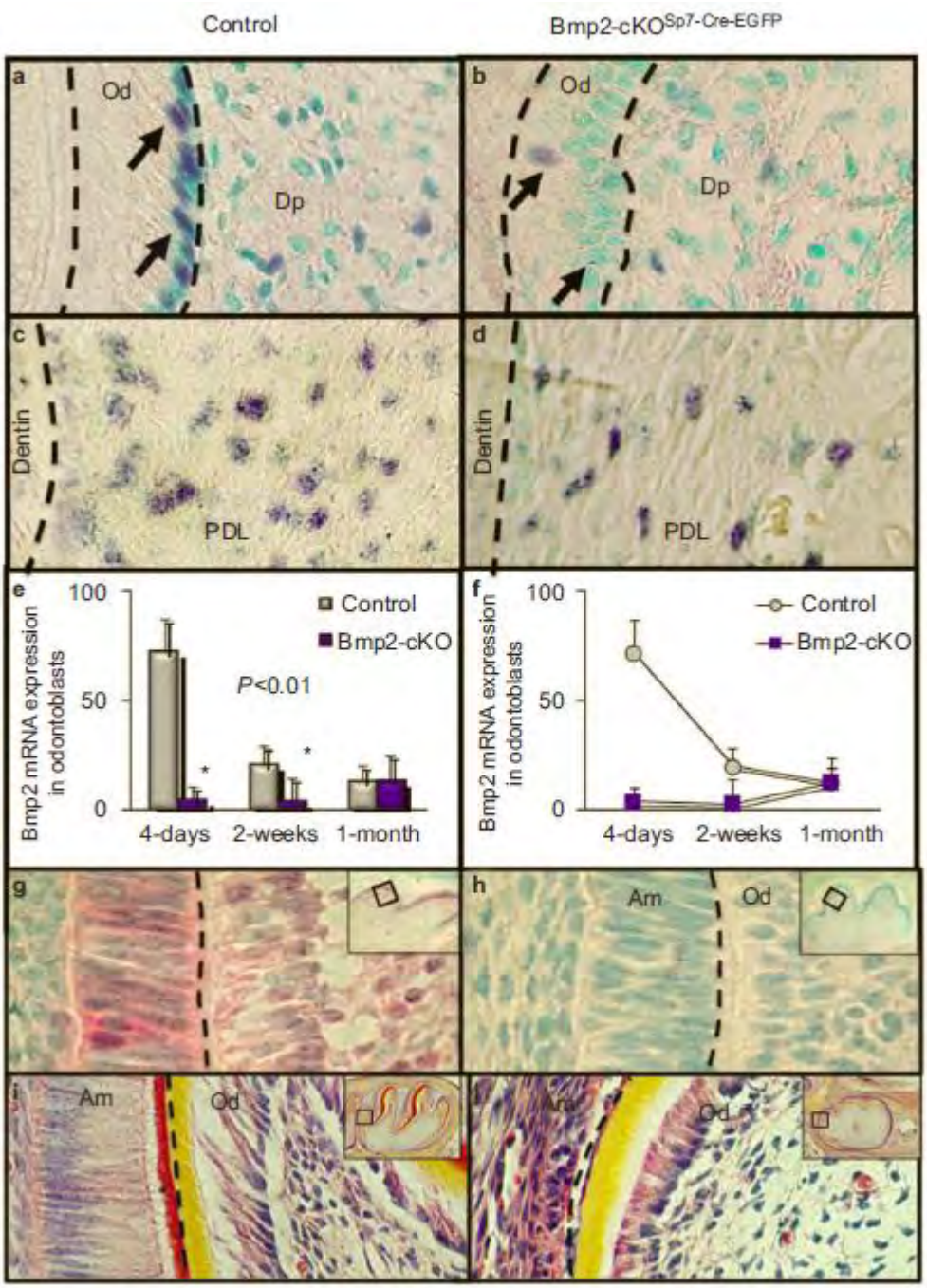


Figure 2 Lineage studies by mapping Cre activity in the root region of a second molar in a 2-week-old mouse by confocal microscopy using the Olympus FV 1000. Combined DAPI-stained nuclei (blue), Sp7-Cre-EGFP$^+$ (green) and tdTomato$^+$ (red) cells are shown. (**a**) DIC image. (**b**) Sp7$^+$ EGFP signal. (**c**) tdTomato signal representing Cre event and cells derived from the Cre events. AP, apical papilla; BV, blood vessel; DAPI, 4′,6-diamidino-2-phenylindole; DIC, differential interference contrast; Dp, dental-pulp chamber; Od, odontoblasts; PDL, periodontal ligament region.

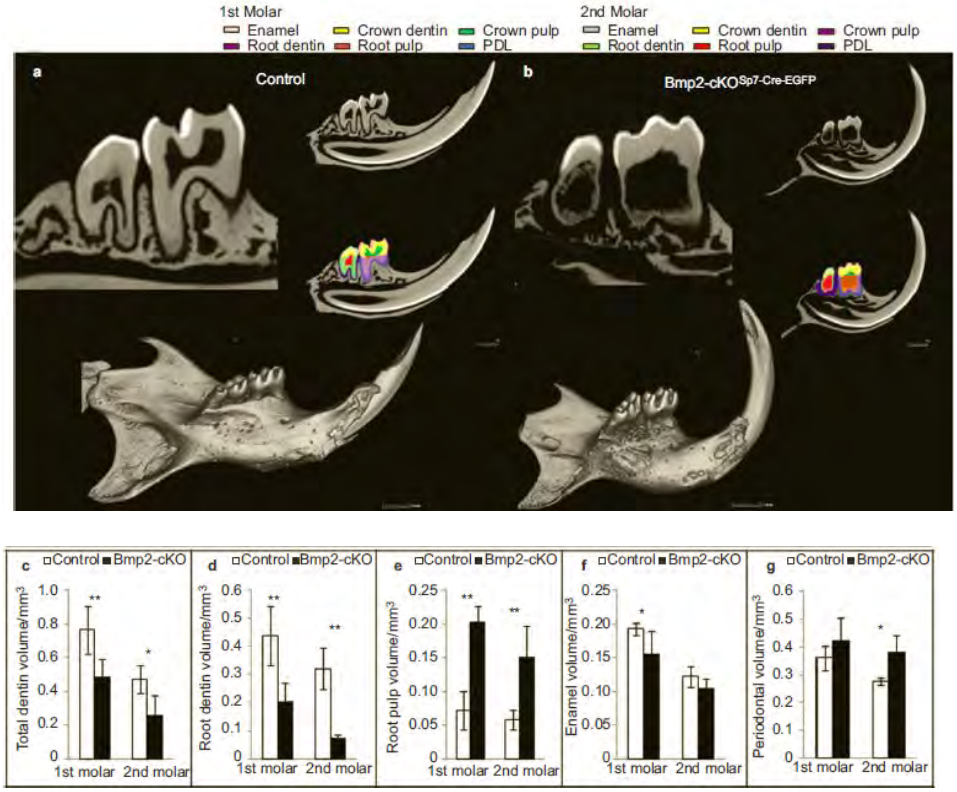
2.Phenotype of the Bmp2-cKO$^{\text{Sp7-Cre-EGFP}}$ mice in the tooth and supporting structures

Phospho-Smad 1/5/8

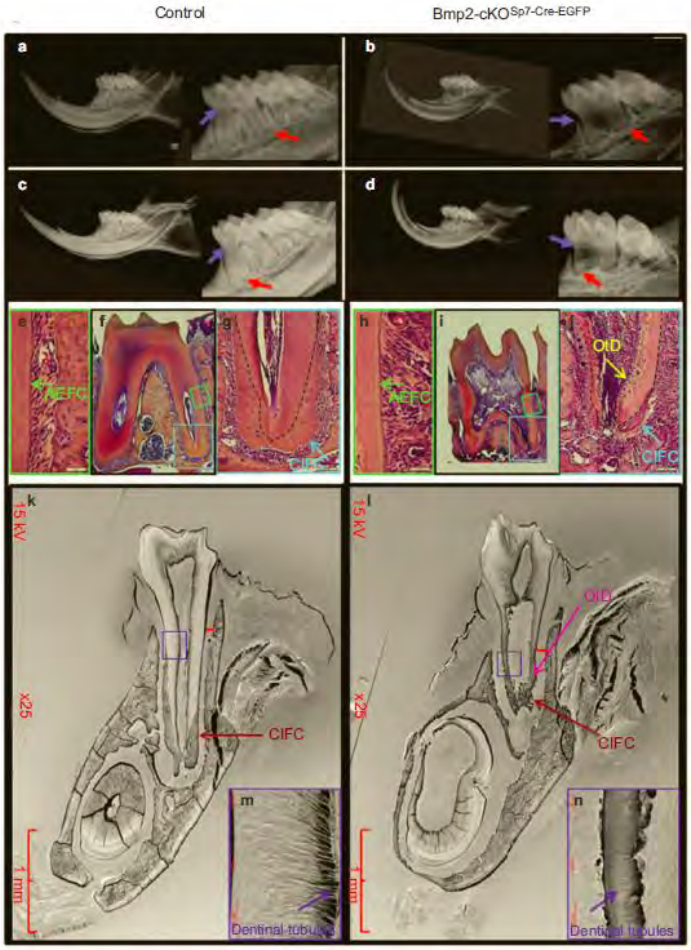

Control
Bmp2-cKO Sp7-Cre-EGFP
a
Od
Dp
b
Od
Dp
c
Dentin
PDL
d
Dentin
PDL
e
Bmp2 mRNA expression in odontoblasts
100
50
0
Control
Bmp2-cKO
P<0.01
4-days
2-weeks
1-month
f
Bmp2 mRNA expression in odontoblasts
100
50
0
Control
Bmp2-cKO
4-days
2-weeks
1-month
g
h
Am
Od
i
Am
Od
j
Am
Od

<Fig3>

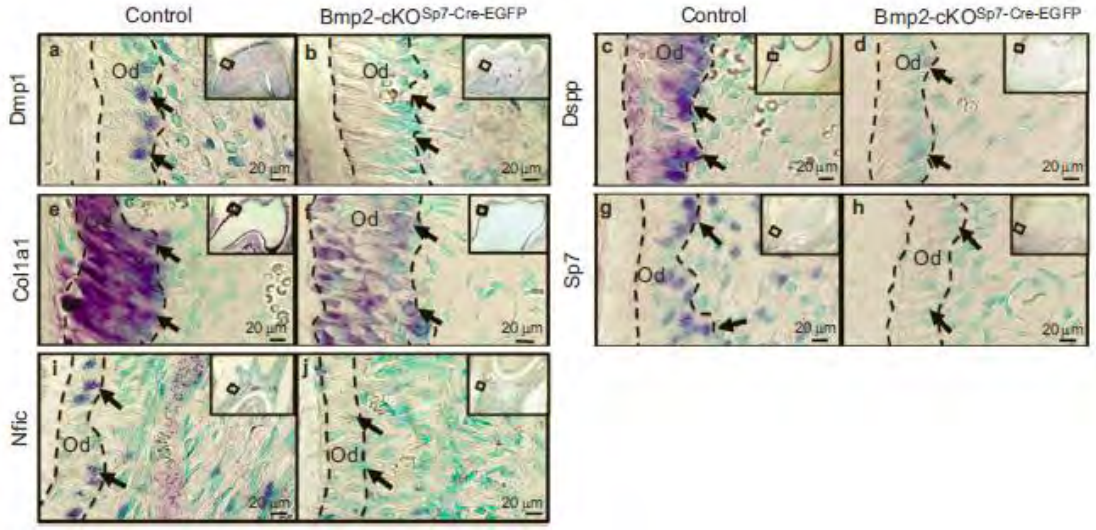
<Fig4>


1st Molar
Enamel
Crown dentin
Crown pulp
Root dentin
Root pulp
PDL
2nd Molar
Enamel
Crown dentin
Crown pulp
Root dentin
Root pulp
PDL
a
Control
b
Bmp2-cKOSp7-Cre-EGFP
c Control Bmp2-cKO
Total dentin volume/mm3
1st molar 2nd molar
d Control Bmp2-cKO
Root dentin volume/mm3
1st molar 2nd molar
e Control Bmp2-cKO
Root pulp volume/mm3
1st molar 2nd molar
f Control Bmp2-cKO
Enamel volume/mm3
1st molar 2nd molar
g Control Bmp2-cKO
Periodontal volume/mm3
1st molar 2nd molar

<Fig 5>

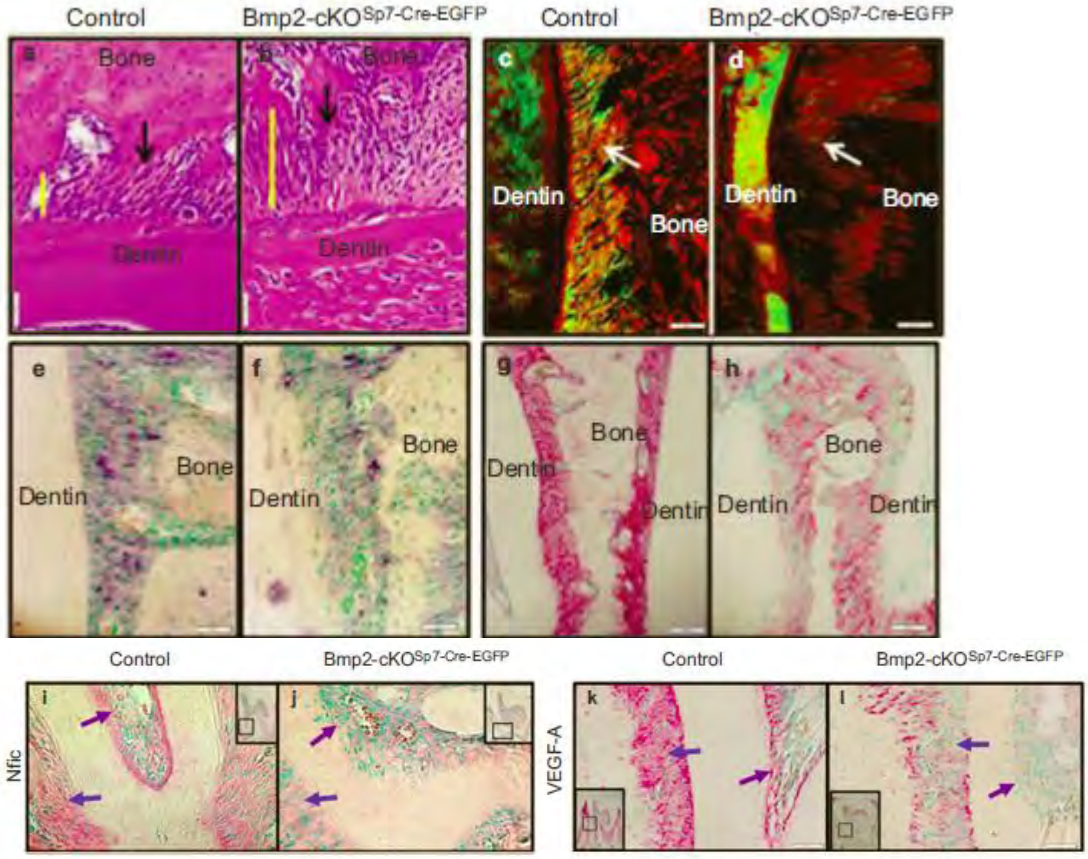

Control
Bmp2-cKOSp7-Cre-EGFP
a
b
c
d
e
f
g
h
i
j
AEFC
OID
CIFC
k
l
15 kV
x25
CIFC
OID
m
n
1 mm
Dentinal tubules

<Fig 6>



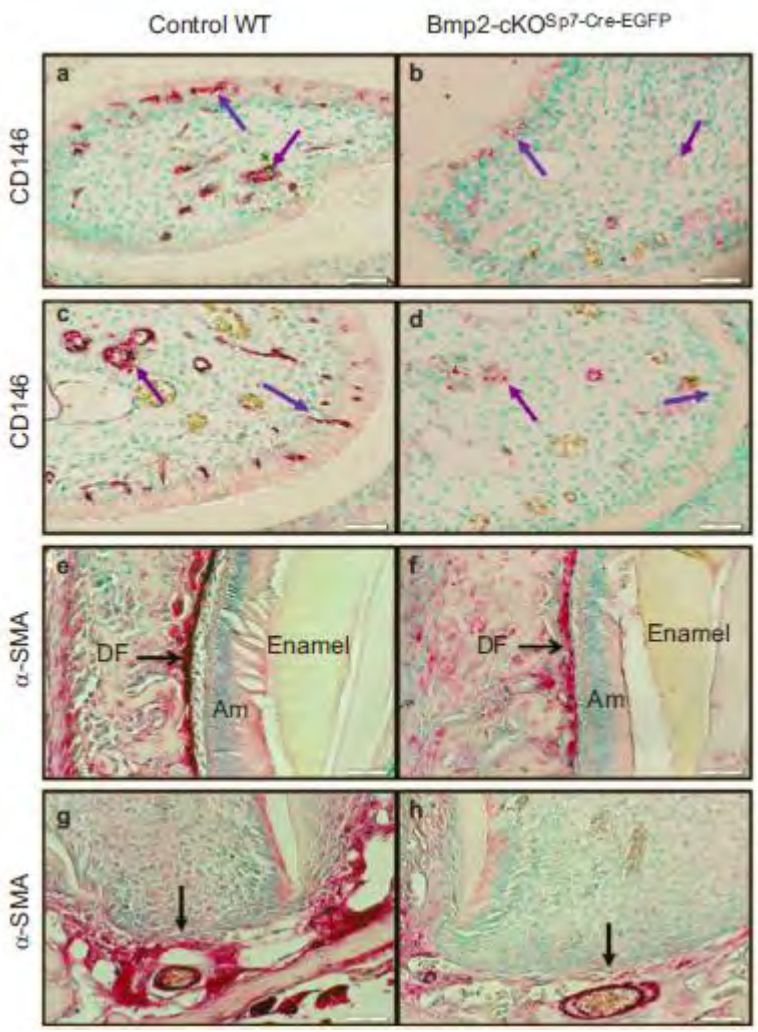
3.Cell proliferation and apoptosis assays in the Bmp2-cKOSp7-Cre-EGFP model

<Fig 7> van Gieson stain Picrosirius Red stain



4.Possible mechanism of Bmp2 action in tooth and supporting structure development
<Fig 8>

Periostin



***DISCUSSION:**

1. α-SMA$^+$ cells are found not only on many of the smooth muscle cells of the larger blood vessels, but also on the smaller microvessels or capillary walls, sometimes referred to as pericytes.
2. α-SMA$^+$ cells are also scattered throughout the odontoblast layer and in the pulp region, not necessarily on microvessels, and are highly expressed in the periodontium.
3. We propose the α- SMA$^+$ cells, at least a subset, are stem cells for odontoblasts, and for
other components of the supporting structures of the teeth, such as cementoblasts, PDL fibroblasts and alveolar bone osteoblasts.
4. Massive osteodentin in the pulp of the Bmp2-cKO$^{Sp7\text{-}Cre\text{-}EGFP}$ mice
→alterations and cell autonomous dysmorphic differentiation of odontoblasts.
5. Recent data have shown that conditional removal of the Bmp2 induced transcription factor, Sp7, leads to major defects in cementogenesis.
6. When the Bmp2 gene is deleted in these several cell types or states, we have shown major defects in tooth root formation, as well as cementum, PDL and alveolar bone formation.
→mechanism: failure to form the vascular systemin the dental pulp and in the periodontium in the absence of the Bmp2 gene in odontoblast and periodontium precursors.
7. Supporting evidence for this hypothesis is presented with the decrease in the

CD146^{+} candidate stem cells on the small microvessels in the dental pulp, and decreased VEGF-A production when the Bmp2 gene is removed from Sp7^{+} cells.
8. We hypothesize that the failure in formation of these α-SMA^{+} cells and/or CD146^{+} cells is linked to the failure to form a vascular niche. Moreover, without sufficient Bmp2 in the early stages of odontoblasts, the root and crown odontoblasts fail to terminally differentiate.
*Summary
When the Bmp2 gene is removed from Sp7^{+} (Osterix^{+}) cells:
1) There are major cell autonomous defects in root-odontoblast terminal differentiation.
2) There are major alterations in formation of the PDLs and cellular cementum, correlated
with decreased nuclear factor IC (Nfic), periostin and a-SMA^{+} cells.
3) There is a failure to produce vascular endothelial growth factor A (VEGF-A) in the periodontium and the pulp leading to decreased formation of the microvascular and associated candidate stem cells in the Bmp2-cKO$^{Sp7\text{-}Cre\text{-}EGFP}$.
4) Ameloblast function and enamel formation are indirectly altered in the Bmp2-cKO$^{Sp7\text{-}Cre\text{-}EGFP}$.
-A critical role for Bmp2 gene in formation and coordination of both the tooth root and supporting structures, including alveolar bone, CIFC and the periodontal ligaments within the periodontium.

題號	題目
1	在上顎竇提升手術中可誘導新生骨形成之 BMP 有 1)BMP-6. 2)BMP-2. 3)BMP-4. 4)BMP-7 (A) 1.2 (B) 2.3 (C) 3.4 (D) 2.4
答 案 (D)	出處：牙周病專科醫師考題
題號	題目
2	骨形成蛋白 BMPs (bone morphogenetic protein).主要是對於調節骨生成.骨維持和修復扮演重要的角色.下列何者不是目前廣泛應用在組織工程上的骨形成蛋白
	(A) BMP-2 (B) BMP-3 (C) BMP-7 (D) BMP-10
答 案 (D)	出處：牙周病專科醫師考題