FOS rearrangement and expression in cementoblastoma

Lam, S.W.; Cleven, A.H.G.; Briaire-de Bruijn, I.H.; Schreuder, W.H.; Kroon, H.M.; Savci-Heijink, D.C.; ... ; Baumhoer, D.

FOS Rearrangement and Expression in Cementoblastoma

Suk Wai Lam, MD, Arjen H.G. Cleven, MD, PhD,* Inge H. Briaire-de Bruijn, MSc,* Willem H. Schreuder, MD,† Herman M. Kroon, MD, PhD,‡ Dilara C. Savci-Heijink, MD, PhD,§ Albert J.H. Suurmeijer, MD, PhD,‖ Karoly Szuhai, MD, PhD,¶ Judith V.M.G. Bovée, MD, PhD,* and Daniel Baumhoer, MD, PhD#*

Abstract: Cementoblastomas are rare odontogenic tumors developing in close proximity to the roots of teeth. Due to their striking morphologic resemblance to osteoblastomas of the peripheral skeleton, we set out to determine whether cementoblastomas harbor the same *FOS* rearrangements with overexpression of c-FOS as has recently been described for osteoblastomas. In total, 16 cementoblastomas were analyzed for FOS expression by immunohistochemistry and for *FOS* rearrangements by fluorescence in situ hybridization (FISH). We observed strong and diffuse staining of c-FOS in 71% of cementoblastomas and identified a *FOS* rearrangement in all cases (n = 3) applicable for FISH. In the remaining cases, FISH failed due to decalcification. Cementoblastomas harbor similar *FOS* rearrangements and show overexpression of c-FOS like osteoblastomas, suggesting that both entities might represent parts of the spectrum of the same disease.

Key Words: cementoblastoma, FOS, bone tumor

(*Am J Surg Pathol* 2021;00:000–000)

Cementoblastoma is a benign odontogenic tumor intimately associated with the roots of teeth.[1,2] It is rare and accounts for 1% to 6% of all odontogenic tumors. Patients show a mean age of 20.7 years.[2] The mandibular molars and premolars are most commonly involved.[2] The radiologic appearance is almost pathognomonic with a well-defined radiopaque mass expanding from the root of a tooth obliterating the periodontal space and generally showing a radiolucent rim (Fig. 1A). Cortical expansion and deviation of the adjacent roots can occur as the tumor grows. The histology of cementoblastoma usually shows an immature, dense, and cementum-like matrix formation attached to the root of a tooth, although this is usually not encountered in biopsy specimens. The reversal lines appear irregular and can resemble Paget disease of bone. Activated cementoblasts, which are morphologically indistinguishable from osteoblasts (seen in osteoblastomas), and a well-vascularized fibroblastic stroma surround the lesional matrix. Whereas the central parts are hypocellular and strongly mineralized, the periphery often contains areas strongly resembling osteoblastoma, which were recently shown to harbor recurrent rearrangements of *FOS* or *FOSB*.[3] Expression of c-FOS detected by immunohistochemistry was subsequently reported as a reliable surrogate marker of this aberration and was demonstrated to be present in >70% of osteoid osteomas and osteoblastomas.[3–5] We hypothesized that cementoblastoma might be related to osteoblastoma and therefore could harbor the same genetic aberration.

MATERIALS AND METHODS

We assembled a set of 16 cementoblastomas comprising 12 cases from the University Hospital Basel and 4 cases from the Leiden University Medical Center. All LUMC samples were handled according to the ethical guidelines described in "Code for Proper Secondary Use of Human Tissue in the Netherlands" in a coded (pseudonymized) manner. Ethical approval for the Basel cases was given by the Ethikkommission beider Basel (reference 274/12).

Immunohistochemistry for c-FOS (Cat. #ABE457; EMD Millipore Corporation, Temecula, CA) was performed as described previously.[5] For in situ hybridization, bacterial artificial chromosomes probes were used proximal and distal to *FOS*, as described previously.[4] *FOS* fluorescence in situ hybridization (FISH) was performed for all available cases and scored by S.W.L. and K.S. after correlation with corresponding hematoxylin and eosin and c-FOS immunohistochemistry slides.

RESULTS

The average age of the patients was 21 years (range: 12 to 47 y) and included 5 men (33%) (Table 1). Tumor size ranged from 10 to 35 mm (Table 1). All cases showed abundant matrix formation consisting of immature, hypocellular, and strongly calcified cementum-like tissue rimmed by plump and activated cementoblasts (Figs. 1B, C). The spaces in

From the Departments of *Pathology; ‡Radiology; ¶Cell and Chemical Biology, Leiden University Medical Center, Leiden; †Department of Oral and Maxillofacial Surgery/Head and Neck Surgery, Amsterdam University Medical Center/Antoni van Leeuwenhoek Hospital; §Department of Pathology, Academic Medical Center, Amsterdam; ‖Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; and #Bone Tumour Reference Centre, Institute of Pathology, University Hospital Basel, University of Basel, Basel, Switzerland.

Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence: Arjen H.G. Cleven, MD, PhD, Department of Pathology, Leiden University Medical Center, PO Box 9600, L1-Q, Leiden 2300 RC, The Netherlands (e-mail: a.h.g.cleven@lumc.nl).

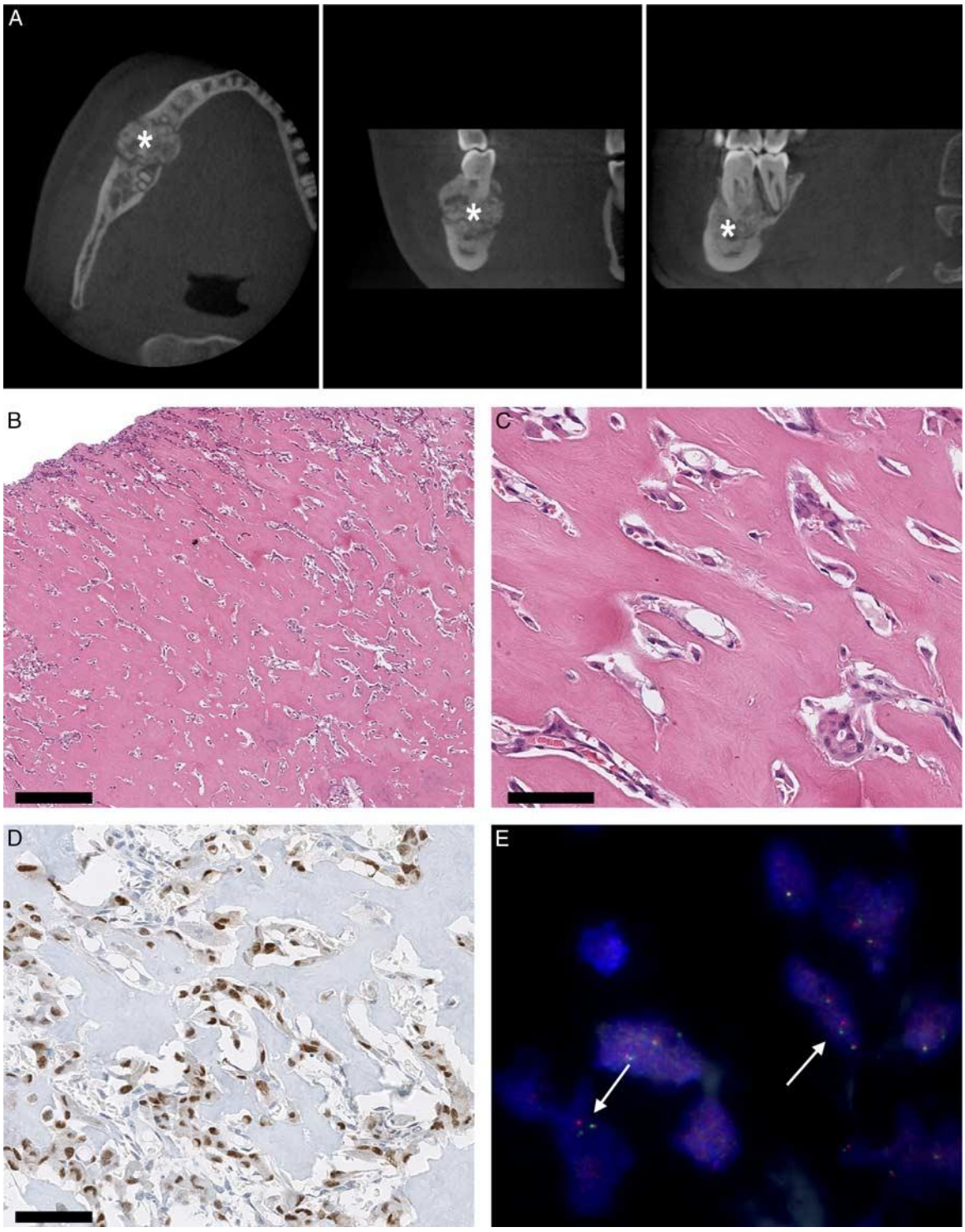

FIGURE 1. Radiology, morphologic findings, c-FOS expression, and FOS rearrangement in cementoblastoma. A, Computed tomography images show a well-defined lesion (~2 cm) in the mandible on the right side (asterisks). The mass demonstrates an ossifying matrix and is closely related to the root of element 4.6 which shows erosion. There is a cortical interruption on both the buccal as well as the lingual side of the mandible. B, Immature and strongly mineralized matrix formation attached to the root of a tooth. C, Activated cementoblasts and a well-vascularized fibrotic stroma surrounding the lesional bone matrix. D, Strong c-FOS nuclear and cytoplasmic expression in lesional cementoblasts intermingled with negative normal cells. E, FISH using split-apart probes for *FOS* shows a segregated red and green signal in cementoblastoma, indicating a *FOS* rearrangement (arrows).

TABLE 1. Overview of Clinical Characteristics and Results of c-FOS Immunohistochemistry and FISH in Our Series of Cementoblastoma

Case	Sex	Age (y)	Tumor Size (mm)	c-FOS IHC	FOS FISH
Basel 1	Female	32	19	Negative	Failed
Basel 2	Female	25	23	Negative	Failed
Basel 3	Female	24	17	NA	Failed
Basel 4	Female	47	17	NA	Failed
Basel 5	Female	12	15	Negative	Failed
Basel 6	Male	22	28	Negative	Failed
Basel 7	Female	16	17	Positive	Positive
Basel 8	Male	13	21	Positive	Failed
Basel 9	Female	13	32	Positive	Failed
Basel 10	Female	14	10	Positive	Positive
Basel 11	Male	22	35	Positive	Failed
Basel 12	Female	12	34	Positive	Failed
LUMC 1	Male	16	25	Positive	Failed
LUMC 2	Female	20	30	Positive	Failed
LUMC 3	Female	13	15	Positive	Failed
LUMC 4	Male	19	16	Positive	Positive

IHC indicates immunohistochemistry; NA, not applicable.

between were occupied by a monomorphic and densely vascularized fibroblastic stroma lacking cytologic atypia. All tumors were sharply delineated and demonstrated the obliteration of the periodontal ligament space by lesional matrix.

We observed strong and diffuse staining of c-FOS in 71% of cases (10/14, Table 1, Fig. 1D), which is in concordance with the expression observed in osteoblastomas ranging from 57% to 83%.[5] All positive cases in our series showed a strong nuclear expression of c-FOS in >50% of tumor cells. Of note, the tumor cells were intermingled with normal cells such as stromal cells and osteoclast-like giant cells.

In 3 cases of cementoblastoma with strong c-FOS expression, we were able to identify a *FOS* rearrangement by FISH (Table 1, Fig. 1E). This is in line with the observed correlation of c-FOS overexpression and *FOS* rearrangements in osteoblastomas.[4,5] Notably, due to varying amounts of intermingled non-neoplastic cells, the percentage of split signals varied between cases, and was in some areas as low as 5% (LUMC case 4). In the residual cases (n = 13) no hybridization signals could be detected, most likely due to aggressive acid decalcification of the tumor samples. In the study by Lam and colleagues, it was furthermore shown that long decalcification times particularly affect c-FOS immunostaining that can result in false-negative results. This mechanism might explain the lack of staining for c-FOS in 4 cases of our study.

DISCUSSION

Since cementoblastomas show a close relationship between the roots of the related teeth, it is believed to originate from cells of the inner dental follicle destined to become cementoblasts,[6] while osteoblastomas and osteoid osteomas are supposed to be derived from osteoprogenitor cells present in the entire skeleton.[1] However, this supposed difference in histogenesis is not translated into a different morphology, as cementoblastoma, osteoblastoma, and osteoid osteoma are histologically nearly identical.[7]

It has been hypothesized before that cementoblastomas might primarily develop as "conventional" osteoblastomas in the tooth-bearing areas of the jaws and secondarily become connected to a tooth.[8] Osteoblastomas and cementoblastomas both occur mostly in the second to third decades of life, recommended treatment is similar and compromises complete surgical excision, both entities may recur following incomplete removal. Here, we demonstrate that both lesions share the same molecular pathogenesis based on the presence of *FOS* rearrangements, adding further proof that cementoblastomas and osteoblastomas/osteoid osteomas indeed form a spectrum of the same disease.

FOS belongs to the FOS family of transcription factors that together with the Jun family members form a group of AP-1 proteins which bind to so-called TPA-responsive elements in the promoter and enhancer regions of target genes.[9] Therefore, FOS proteins regulate and influence various biological processes, including cell proliferation, differentiation, and survival. During normal osteoblast maturation, FOS and other members of the FOS family are highly expressed.[10] Similar to osteoblastoma, recurrent rearrangements of *FOS* or *FOSB* are also found in vascular tumors such as *FOS*-rearranged epithelioid hemangioma, and *FOSB* fusions are described in atypical epithelioid hemangioma and pseudomyogenic hemangioendothelioma.[11–14]

Histologically, many features of cementoblastoma can be encountered also in osteoblastoma, a radiologic correlation is, therefore, essential to demonstrate the connection with the root of a tooth in the case of cementoblastoma. The same holds true also for osteosarcoma which generally presents more aggressively on imaging analyses and shows cellular atypia typically lacking in cementoblastoma. The expression of c-FOS alone, however, can be observed also in a smaller subset of osteosarcomas (in 14%) and even in osteoblasts of reactive new bone formation.[4,5]

Since the percentage of actual tumor cells can be very low (exemplified by LUMC case 4) and FISH testing for *FOS* rearrangements often fails due to prior tissue decalcification, the correlation between morphology and radiology remains the cornerstone in the diagnosis of cementoblastomas and its differential diagnoses. In conclusion, our study shows that cementoblastomas not only share morphologic features but also harbor similar *FOS* rearrangements and c-FOS expression like osteoblastomas/osteoid osteomas, suggesting that cementoblastomas are part of the spectrum of the same disease localized at the root of teeth. Although the use of c-FOS immunohistochemistry is limited in its differential diagnosis, confirming the presence of a *FOS* translocation using FISH, whenever possible, can be of aid in diagnostic challenges.

REFERENCES

1. Slootweg PJ. Cementoblastoma and osteoblastoma: a comparison of histologic features. *J Oral Pathol Med*. 1992;21:385–389.
2. Chrcanovic BR, Gomez RS. Cementoblastoma: an updated analysis of 258 cases reported in the literature. *J Craniomaxillofac Surg*. 2017;45:1759–1766.
3. Fittall MW, Mifsud W, Pillay N, et al. Recurrent rearrangements of FOS and FOSB define osteoblastoma. *Nat Commun*. 2018;9:2150.

4. Lam SW, Cleven AHG, Kroon HM, et al. Utility of FOS as diagnostic marker for osteoid osteoma and osteoblastoma. *Virchows Arch*. 2020;476:455–463.
5. Amary F, Markert E, Berisha F, et al. FOS expression in osteoid osteoma and osteoblastoma: a valuable ancillary diagnostic tool. *Am J Surg Pathol*. 2019;43:1661–1667.
6. Ulmansky M, Hjorting-Hansen E, Praetorius F, et al. Benign cementoblastoma. A review and five new cases. *Oral Surg Oral Med Oral Pathol*. 1994;77:48–55.
7. El-Naggar AK, Chan JKC, Grandis JR, et al. *WHO Classification of Head and Neck Tumours*. Lyon, France: International Agency for Research on Cancer (IARC); 2017.
8. Slootweg P. *Pathology of the Maxillofacial Bones: A Guide to Diagnosis*. Cham, Switzerland: Springer International Publishing; 2015:5–6.
9. Milde-Langosch K. The Fos family of transcription factors and their role in tumourigenesis. *Eur J Cancer*. 2005;41:2449–2461.
10. Bozec A, Bakiri L, Jimenez M, et al. Fra-2/AP-1 controls bone formation by regulating osteoblast differentiation and collagen production. *J Cell Biol*. 2010;190:1093–1106.
11. Agaram NP, Zhang L, Cotzia P, et al. Expanding the spectrum of genetic alterations in pseudomyogenic hemangioendothelioma with recurrent novel ACTB-FOSB gene fusions. *Am J Surg Pathol*. 2018; 42:1653–1661.
12. Huang SC, Zhang L, Sung YS, et al. Frequent FOS gene rearrangements in epithelioid hemangioma: a molecular study of 58 cases with morphologic reappraisal. *Am J Surg Pathol*. 2015;39: 1313–1321.
13. Antonescu CR, Chen HW, Zhang L, et al. ZFP36-FOSB fusion defines a subset of epithelioid hemangioma with atypical features. *Genes Chromosomes Cancer*. 2014;53:951–959.
14. van Ijzendoorn DG, de Jong D, Romagosa C, et al. Fusion events lead to truncation of FOS in epithelioid hemangioma of bone. *Genes Chromosomes Cancer*. 2015;54:565–574.