Molecular pathology in bone and soft tissue tumors: a multifunctional key for diagnosis and prediction

Lam, S.W.

Chapter 6

FOS rearrangement and expression in cementoblastoma

Suk Wai Lam, Arjen H.G. Cleven, Inge H. Briaire-de Bruijn, Willem H. Schreuder, Herman M. Kroon, C. Dilara Savci-Heijink, Albert H. Suurmeijer, Karoly Szuhai, Judith V.M.G. Bovée and Daniel Baumhoer

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Abstract

Aim

Cementoblastomas are rare odontogenic tumors developing in close proximity to the roots of teeth. Due to their striking morphological resemblance to osteoblastomas of the peripheral skeleton, we set out to determine whether cementoblastomas harbor the same *FOS* rearrangements with overexpression of FOS as has recently been described for osteoblastomas.

Methods and results

In total, sixteen 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 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.

Conclusion

Cementoblastomas harbor similar *FOS* rearrangements and show overexpression of FOS like osteoblastomas, suggesting that both entities might represent parts of the spectrum of the same disease.

Introduction

Cementoblastoma is a benign odontogenic tumor intimately associated with the roots of teeth [1, 2]. It is rare and accounts for 1-6 % 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 radiological 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. 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 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 osteoblastoma [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 FOS (EMD Millipore Corporation, Temecula, CA, USA, Cat. #ABE457) was performed as described previously [5]. For *in-situ* hybridization, BAC probes were used proximal and distal to *FOS*, as described previously [4]. *FOS* FISH was performed for all available cases and scored by SWL and KZ after correlation with corresponding H&E and FOS immunohistochemistry slides.

Results

The average age of the patients was 21 years (range: 12 to 47y) and included five men (33%). Tumor size ranged from 10-35 mm (**Table 1**). All cases showed abundant matrix formation consisting of immature, hypocellular and strongly calcified

Table 1. Overview of clinical characteristics and results of FOS immunohistochemistry and fluorescence *in-situ* hybridization in our series of cementoblastoma

Case	Gender	Age	Tumor size	FOS IHC	FOS FISH
Basel 1	f	32	19 mm	neg	failed
Basel 2	f	25	23 mm	neg	failed
Basel 3	f	24	17 mm	N/A	failed
Basel 4	f	47	17 mm	N/A	failed
Basel 5	f	12	15 mm	neg	failed
Basel 6	m	22	28 mm	neg	failed
Basel 7	f	16	17 mm	pos	pos
Basel 8	m	13	21 mm	pos	failed
Basel 9	f	13	32 mm	pos	failed
Basel 10	f	14	10 mm	pos	pos
Basel 11	m	22	35 mm	pos	failed
Basel 12	f	12	34 mm	pos	failed
LUMC1	m	16	25 mm	pos	failed
LUMC2	f	20	30 mm	pos	failed
LUMC3	f	13	15 mm	pos	failed
LUMC4	m	19	16 mm	pos	pos

Pos, positive; neg, negative; N/A, not applicable

cementum-like tissue rimmed by plump and activated cementoblasts (**Figure 1B** and **C**). The spaces in between were occupied by a monomorphic and densely vascularized fibroblastic stroma lacking cytologic atypia. All tumors were sharply delineated and demonstrated obliteration of the periodontal ligament space by lesional matrix.

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

In three cases of cementoblastoma with strong FOS expression, we were able to identify a *FOS* rearrangement by FISH (**Figure 1E**). This is in line with the observed correlation of 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% (LUMC4 case). 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 et al. it was furthermore shown that long decalcification times particularly affect FOS immunostaining that can result in false negative results. This mechanism might explain the lack of staining for FOS in four cases of our study.

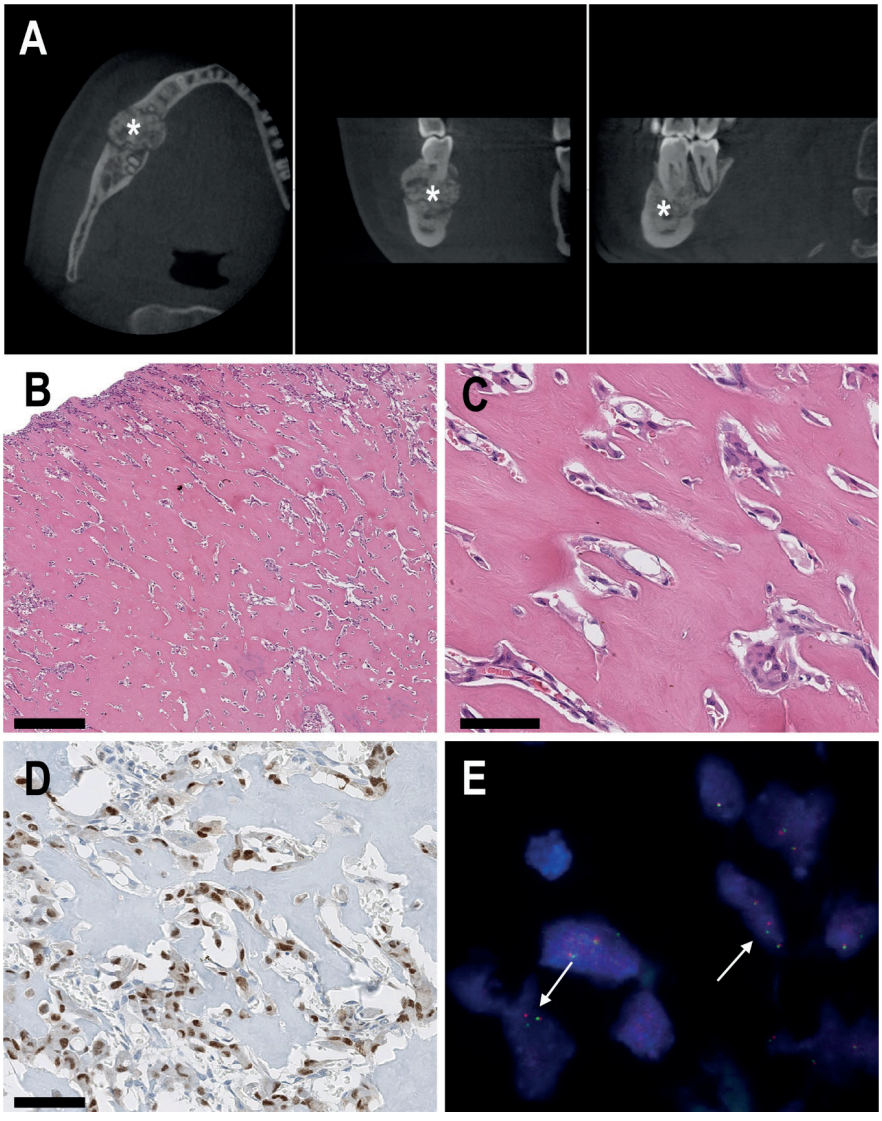

Figure 1. Radiology, morphological findings, FOS expression and *FOS* rearrangement in cementoblastoma. **A**: Computed tomography images show a well-defined lesion (~2cm) in the mandible on the right side (asterisks). The mass demonstrated an ossifying matrix and is closely related to the root of element 4.6 which shows erosion. There is 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 FOS nuclear and cytoplasmic expression in lesional cementoblasts, intermingled with negative normal cells. **E**: Fluorescence *in-situ* hybridization (FISH) using split-apart probes for *FOS* shows a segregated red and green signal in cementoblastoma, indicating a *FOS* rearrangement (arrows).

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 radiological correlation is therefore essential to demonstrate the connection with the root of a tooth in case of cementoblastoma. The same holds true for osteosarcoma which generally presents more aggressively on imaging analyses and shows cellular atypia typically lacking in cementoblastoma.

The expression of 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, 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 morphological features, but also harbor similar *FOS* rearrangements and 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 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, Briaire-de Bruijn IH, Szuhai K, Bovée J. 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, Haque MF. 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, Takata T, Slootweg P. *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. *Pathology of the maxillofacial bones. A guide to diagnosis.* Switserland: 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, Schinke T, Amling M, Wagner EF. 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, Antonescu CR. 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 D, 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.