FOS Expression in Osteoid Osteoma and Osteoblastoma

A Valuable Ancillary Diagnostic Tool

Fernanda Amary, MD, PhD,† Eva Markert, MD,* Fitim Berisha, MSc,* Hongtao Ye, PhD,* Craig Gerrand, MD,* Paul Cool, MD,‡ Roberto Tirabosco, MD,* Daniel Lindsay, MD,* Nischalan Pillay, MD, PhD,*† Paul O'Donnell, MD,* Daniel Baumhoer, MD, PhD,§ and Adrienne M. Flanagan, MD, PhD*†*

Abstract: Osteoblastoma and osteoid osteoma together are the most frequent benign bone-forming tumor, arbitrarily separated by size. In some instances, it can be difficult to differentiate osteoblastoma from osteosarcoma. Following our recent description of *FOS* gene rearrangement in these tumors, the aim of this study is to evaluate the value of immunohistochemistry in osteoid osteoma, osteoblastoma, and osteosarcoma for diagnostic purposes. A total of 337 cases were tested with antibodies against c-FOS: 84 osteoblastomas, 33 osteoid osteomas, 215 osteosarcomas, and 5 samples of reactive new bone formation. In all, 83% of osteoblastomas and 73% of osteoid osteoma showed significant expression of c-FOS in the osteoblastic tumor cell component. Of the osteosarcomas, 14% showed c-FOS expression, usually focal, and in areas with severe morphologic atypia which were unequivocally malignant: 4% showed more conspicuous expression, but these were negative for *FOS* gene rearrangement. We conclude that c-FOS immunoreactivity is present in the vast majority of osteoblastoma/osteoid osteoma, whereas its expression is usually focal or patchy, in no more than 14% of osteosarcoma biopsies. Therefore, any bone-forming tumor cases with worrying histologic features would benefit from fluorescence in situ hybridization analysis for *FOS* gene rearrangement. Our findings highlight the importance of undertaking a thorough assessment of expression patterns of antibodies in the light of morphologic, clinical, and radiologic features.

Key Words: bone tumor, osteoblastoma, osteoid osteoma, FOS, c-FOS, immunohistochemistry, FISH, osteosarcoma

Osteoid osteoma and osteoblastoma together are the most common bone-forming tumors. Arbitrarily separated by size, osteoid osteoma is defined as measuring <2 cm diameter, and usually with a classic clinical presentation of nocturnal pain that is relieved with the use of non–steroidal anti-inflammatory drugs.[1,2] Osteoblastoma is less common than osteoid osteoma and represents 1% of all benign bone tumors, being by definition 2 cm or more in diameter and frequently slow-growing.[3,4] Both lesions are usually well-defined on imaging.[5]

In the absence of perilesional sclerosis, often seen in intracortical osteoid osteomas, osteoid osteoma, and osteoblastoma are frequently histologically indistinguishable and characterized by interconnecting trabeculae of woven bone rimmed by plump osteoblasts. The stroma is usually highly vascular with fibroblastic spindle cells and osteoclast-like giant cells.[2,3,6] Although in many clinical scenarios both lesions are fairly easily recognized, in some instances, the differential diagnosis may be challenging. This could be due to the histologic and radiologic appearances, sample size, or even absence of clinical and radiologic information.[2] As bone-forming tumors, the main differential diagnosis is osteosarcoma.

In some instances it may be difficult, and occasionally impossible to differentiate osteoblastoma from the so-called osteoblastoma-like osteosarcoma.[7,8] The controversial concept of the histologically labelled "aggressive osteoblastoma" also plays a role in the differential diagnosis of unusual cases. Aggressive osteoblastoma was initially defined as an osteoblastoma with a distinct epithelioid morphology and therefore also referred to as epithelioid osteoblastomas.[9] However, not all epithelioid osteoblastomas are clinically aggressive. Furthermore, some nonepithelioid osteoblastomas, usually larger than 4 cm, are associated with bone destruction and locally aggressive behavior which adds to the controversy around the term "aggressive" osteoblastoma.[10] To compound the diagnostic challenge, rare cases of osteoblastomas transforming into osteosarcomas have been reported.[11,12]

Until recently, ancillary methods provided little help in terms in differentiating benign from malignant bone-forming

tumors. Our group, however, identified the structural rearrangements involving *FOS* and rarely *FOSB* in osteoblastomas and osteoid osteoma by whole genome, exome, and RNA sequencing.[13] The results were validated in a larger cohort using break-apart fluorescence in situ hybridization (FISH) probes flanking these genes.[13,14]

FOS and *FOSB* are members of the activated protein-1 family of transcription factors. The role of c-FOS/c-Jun pathway has been of significant interest to researchers investigating osteosarcoma and chondrosarcoma as c-*FOS* was identified as an oncogenic element of the FBJ murine osteosarcoma virus in the development of osteosarcoma. The importance of the *FOS* gene in osteosarcoma was underscored when primary bone sarcomas developed in transgenic mice as a result of FOS overexpression.[15–19]

Herein we assessed the pattern of c-FOS expression in a cohort of osteoblastoma and osteoid osteoma from 3 institutions. We also assessed its diagnostic value by analyzing c-FOS expression in a separate cohort of biopsy samples of consecutive osteosarcoma cases.

MATERIALS AND METHODS

Sample Selection

Cases were identified by searching the histopathology files at the Royal National Orthopaedic Hospital (RNOH), The Robert Jones and Agnes Hunt Orthopaedic Hospital (RJAH), and the Basel Bone Tumour Reference Centre, Switzerland. The RNOH Biobank was approved by the National Research Ethics Committee of the Health Research Committee (reference 15/YH/0311: Integrated Research Application System [IRAS] project identifier: 18309). This specific project was approved by the National Research Ethics Committee approved the UCL/UCLH Biobank Ethics Committee (specific project reference no. EC17.14). This biobank was licensed by the Human Tissue Authority under number 12073. Cases from the RJAH biobank were approved by the National Research Ethics Committee of the Health Research Committee (reference 17/YH/0108; Integrated Research Application System [IRAS] project identifier: 217446 and licensed by the HTA under number 12073). Ethical approval was also given by the Ethikkommission bei der Basel (reference 274/12).

Hematoxylin and eosin–stained slides of osteoblastomas and osteoid osteomas were reviewed by specialist bone tumor pathologists (A.M.F., D.B., E.M., F.A., R.T.). Biopsies of consecutive cases of osteosarcomas diagnosed between 2015 and 2018 were also included. Samples reported as osteoblastoma-like osteosarcoma, osteosarcomas from the spine and the jaws were retrieved from outside this time period and included in the study. Cases were excluded when material were not available for additional tests. Clinical and radiological data were included when available. All samples had been fixed in 10% formal saline, decalcified in EDTA or in nitric acid (5%) and processed in paraffin.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections using the monoclonal antibody: anti-c-FOS targeting the N-terminus (ABE457, Merck Millipore, MA). Reactions were performed using the Leica Bond III automated immunostaining platform, with peroxidase blocking, and detection carried out using the Leica Bond Polymer Refine DAB kit (Leica, DS9800; Leica Microsystems, Milton Keynes, UK) according to the manufacturer's instructions: pretreatment: Leica Epitope Retrieval solution 1 (AR9961) for 20 minutes. Dilution: 1/700 Leica Bond Primary Antibody Diluent (Leica, AR9352) for 30 minutes at ambient temperature. The expression was analyzed in the osteoblastic cell component and scored as nuclear expression in <10% or 10% or more of the osteoblastic cell component.

Fluorescence In Situ Hybridization

FISH was performed as described previously.[20] In brief, deparaffinized sections were pretreated by pressure-cooking and incubated in pepsin solution at 37°C for 50 minutes. Probes were added to tissue sections, denatured at 72°C and hybridized overnight at 37°C. Thereafter, the sections were washed and counterstained with 4′, 6-diamidino-2-phenylindole (DAPI) and mounted with coverslips.

FISH was performed in all cases exhibiting no FOS expression by immunohistochemistry. Dual color break-apart Agilent SureFISH custom-designed probes (Agilent, Cheshire, UK) flanking *FOS* and *FOSB* were used. Controls validated by RNA sequencing in our previous study[13] were used in each run. A minimum of 50 nonoverlapping nuclei per case were assessed for break-apart signals.

RESULTS

A total of 337 cases were analyzed by c-FOS immunohistochemistry: 84 osteoblastoma, 33 osteoid osteomas, 215 biopsies of osteosarcoma, and 5 samples of reactive new bone formation. In the osteoblastoma group of 84, the age of presentation ranged from 2 to 61 years old (mean: 21 y). Eighty percent (n = 67) were under 30 years of age with a sex ratio of ~2M:1F (59M:25F). The most frequent site involved was the spine (n = 31; 37%), followed by long tubular bones (n = 17; 20%), feet (n = 13; 15%), bones of the jaw (n = 9; 11%), and pelvis (n = 8; 10%). Two or fewer cases occurred at each of the following sites: bone of the hands, scapula, skull, and ribs.

The 33 osteoid osteoma samples were from patients aged between 5 and 52 years old (mean: 20 y), with a sex ratio of 2M:1F. Fifteen occurred in long tubular bones, 12 in the spine, 5 in the bones of the hands or feet, and 1 in the pelvis.

Among the osteosarcoma cohort, the age of presentation ranged between 2 and 87 years (average: 26); 1.3M:1F. 124 occurred in the long tubular bones, 20 in the spine, and 23 in the jaw bones. Other sites varied and are shown in the Supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/PAS/A836) as well as type of tissue sample tested.

The clinical information on the 5 cases of reactive new bone formation are displayed in the Supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/PAS/A836).

c-FOS Immunohistochemistry

The majority of osteoblastomas (70/84; 83%) and osteoid osteomas (24/33; 73%) showed nuclear expression of c-FOS. The expression was, in most cases, easily appreciated in the plump osteoblasts (Fig. 1 and Table 1). The stromal fibroblastic spindle cells, osteoclast-like giant cells and endothelial cells were consistently negative. Five of 18 negative osteoblastomas and osteoid osteomas with surplus tissue available showed that the blood vessels did not express CD31 thereby demonstrating that tissue processing, most likely due to decalcification, impaired antigenicity in <4% of the cases (Supplemental Table 1,

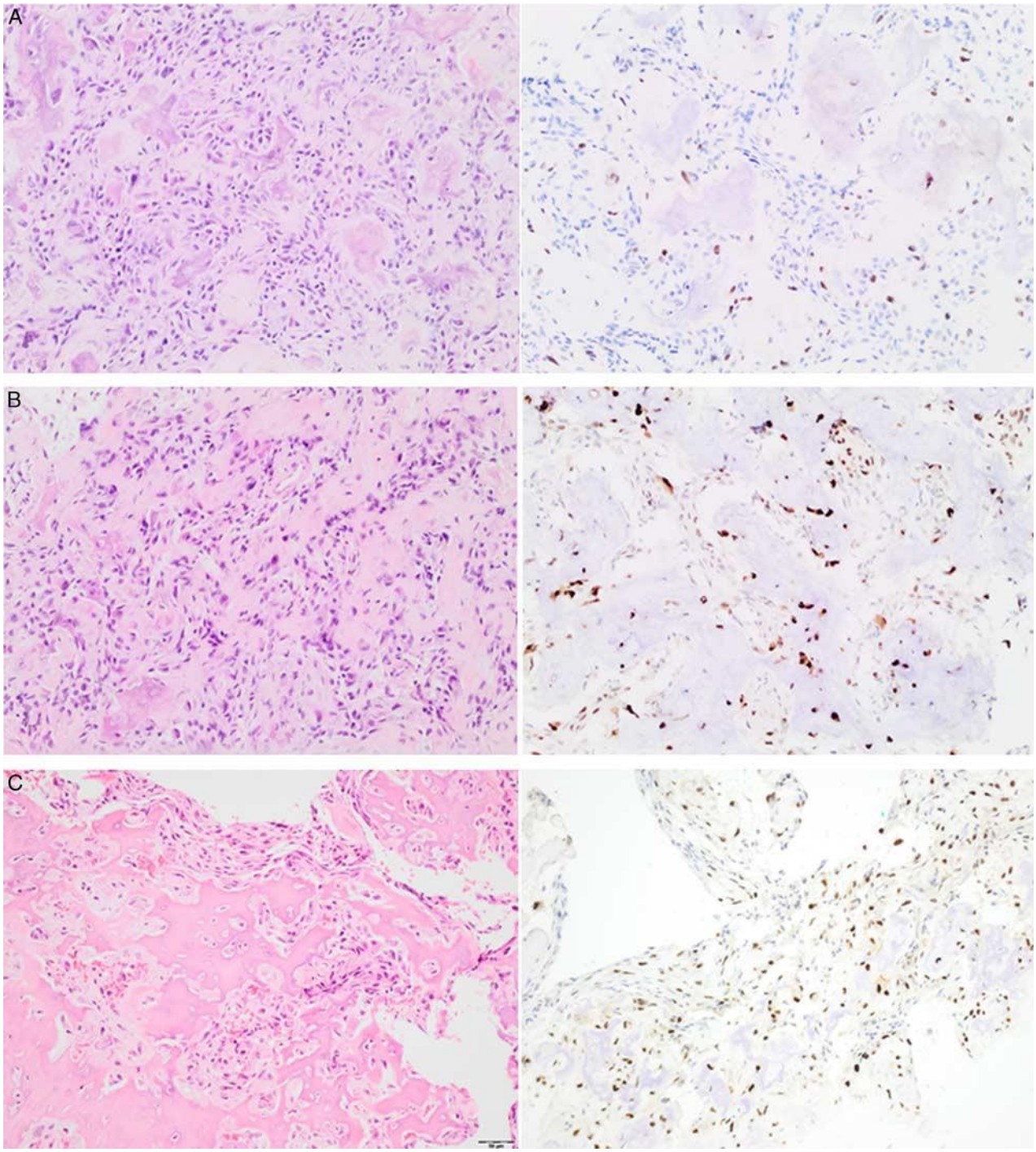

FIGURE 1. Photomicrographs of 3 different cases of osteoblastoma/osteoid osteoma (A–C) demonstrating the histological features and corresponding c-FOS expression limited to the plump osteoblastic cells. The stromal fibroblastic cells, endothelial cells and osteoclast-like giant cells are consistently negative for c-FOS.

TABLE 1. Summary of c-FOS Immunohistochemistry

	No. of Cases Showing c-FOS Expression (%)	% of Tumor Cells	No. of Cases
Osteoid osteoma	24 (73)	< 10 (+)	5
		10-50 (++)	8
		> 50 (+++)	11
Osteoblastoma	70 (83)	< 10 (+)	6
		10-50 (++)	38
		> 50 (+++)	26
Osteosarcoma	31 (14)	< 10 (+)	23
		10-50 (++)	7
		> 50 (+++)	1

Supplemental Digital Content 1, http://links.lww.com/PAS/A836).

Of the osteosarcomas studied, 14% (31/215) showed some degree of nuclear c-FOS immunoreactivity. In most cases the expression was focal, occurring in <10% of the tumor cells and the immunoreactivity was observed predominantly in nonosteoblastic areas (Figs. 2A–C). Eight cases showed more diffuse expression, which in some cases included bone-forming areas, as exemplified in a radiation-induced osteosarcoma of the spine (Fig. 2D).

Of the 4 cases diagnosed as osteoblastoma-like osteosarcoma, only one was diffusely immunoreactivity for c-FOS. This tibial tumor which had extraosseous extension was highly cellular. It was initially labelled as osteoblastoma, but following local recurrence it was sent to a number of external reviewers for their opinion. It was considered a challenging case but on balance the consensus was that the tumor would be best classified as osteoblastoma-like osteosarcoma, a diagnosis that was supported by the radiological aggressive features. The patient did not develop metastatic disease and is alive with no signs of recurrence 11 years after the initial presentation (Fig. 3, Supplemental Fig. 1, Supplemental Digital Content 2, http://links.lww.com/PAS/A837).

The 5 cases of reactive bone formation analyzed were negative for c-FOS.

FOS and *FOSB* FISH

All samples of osteoblastoma and osteoid osteoma that were negative for c-FOS on immunohistochemistry were tested for *FOS* and *FOSB* gene rearrangements by FISH. Of the 14 osteoblastomas negative for c-FOS, 6 were negative for rearrangement by FISH, 7 were noninformative, and 1 (epithelioid osteoblastoma) was a consultation case with no extra slides available for FISH analysis. Of the 9 c-FOS negative osteoid osteomas, 2 showed *FOS* gene rearrangement by FISH, 5 were negative for *FOS* and *FOSB* gene rearrangement and 2 were noninformative. None showed copy number gain or loss.

Of the 215 osteosarcoma cases studied for c-FOS immunoreactivity, 31 (14%) showed some degree of c-FOS expression, and 8 cases showed a diffuse expression pattern (over 10% of the cells). These cases were tested by FISH and only 1, the osteoblastoma-like osteosarcoma, described above, which was diffusely immunoreactive for c-FOS, showed a *FOS* gene rearrangement. Four cases were negative for a *FOS* gene rearrangement but showed multiple copies of the *FOS* locus. There was no tissue available for FISH analysis on the remaining 3 cases.

DISCUSSION

In this study we demonstrate that c-FOS has a distinctive pattern of protein expression in the majority of osteoblastomas and osteoid osteomas,and establish that c-FOS immunohistochemistry can be employed as a useful marker in the diagnoses of these tumor types. Furthermore, the similar pattern of expression seen in both these tumor types, frequently morphologically identical, supports the genetic findings that they represent a spectrum of the same disease.[21,22]

Although the vast majority of osteosarcomas are essentially negative for c-FOS protein expression, immunoreactivity was observed in 14% of the cases: this was usually focal and in non–bone-forming areas, and in unequivocally high grade lesions. A minority of osteosarcomas (< 4%), however, showed a more conspicuous expression of c-FOS in over 10% of the cells. Therefore, immunohistochemistry results must be interpreted in the light of appropriate clinical, morphologic, and radiologic information by an experienced bone pathologist. Whether some of these osteosarcomas have arisen on the background of a benign lesion, a concept not fully accepted in the literature,[11,12] or whether the expression is driven by other mechanisms in these tumors is not clear. The latter is more likely, as the gene rearrangement involving *FOS* has not been detected in the osteosarcomas in which we observed c-FOS immunoreactivity. Furthermore, no *FOS* gene rearrangement was reported in over 55 cases subjected to WGS reviewed by our group in collaboration with the Wellcome Trust Sanger Institute.[23–25]

c-FOS expression was reported in osteosarcomas over 2 decades ago, although the antibodies used in these studies potentially recognized epitopes within the protein other than those using the current antibody.[15–17] The antibody used in the current study target the N-terminus, present in the truncated c-FOS protein as a result of the rearrangement, similar to the mechanism described in cases of epithelioid hemangiomas harboring *FOS* gene rearrangement with breakpoints in the same exon 4 as described in osteoblastomas/osteoid osteomas.[13,22]

It is of particular interest that that the case classified as osteoblastoma-like osteosarcoma in our study (see above), which was strongly positive for c-FOS, harbored a *FOS* gene rearrangement. This case had been diagnosed initially as an aggressive osteoblastoma and following the recurrence was sent for external review to 2 other bone tumor centers. The consensus, at that time, was to classify this lesion as an osteoblastoma-like osteosarcoma. In view of the current finding of the *FOS* gene rearrangement, and a 10-year disease-free follow-up, on hindsight, this case is likely to represent an aggressive (epithelioid) osteoblastoma. This case highlights the value of FISH as a diagnostic adjunct in cases where the differential diagnosis lies between an osteoblastoma and an osteosarcoma. All other osteosarcoma cases that were informative by FISH and in which over 10% of the cells ex-

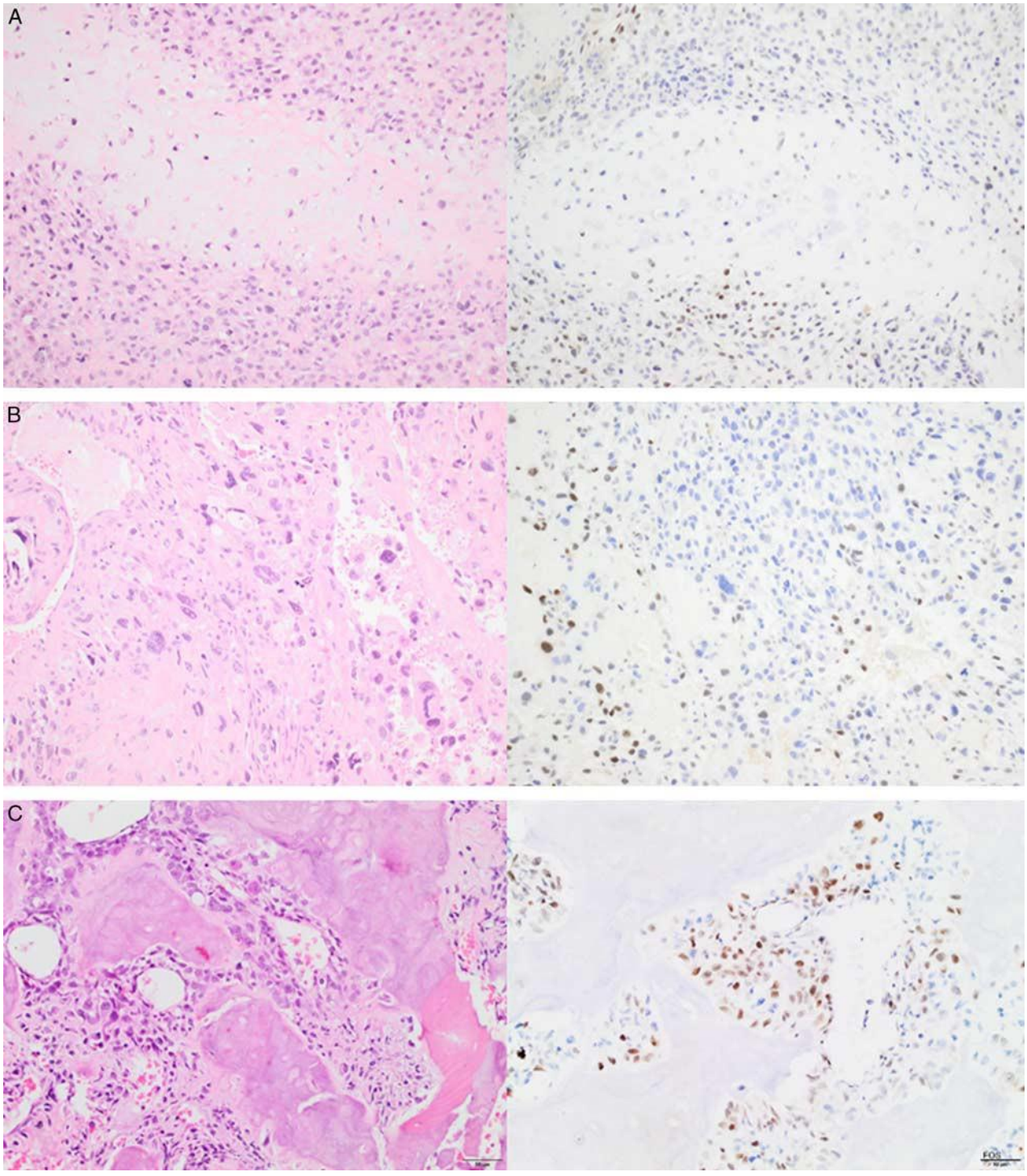

FIGURE 2. Photomicrographs of 3 different cases of osteosarcoma (A–C) showing different patterns of c-FOS expression (right panel).

pressed c-FOS, were all negative for a *FOS* gene rearrangement. Furthermore, all of these cases revealed multiple copies of the *FOS* locus reflecting the polyploid/aneuploidy nature of most osteosarcomas. As stated in our previous study, whole genome sequencing of osteoblastoma generally shows a quiet pattern with very few alterations, in marked distinction to the "chaotic" circus plots seen in osteosarcomas.

Finally, aggressive osteoblastoma is a controversial concept, classically described as a predominantly epithelioid, mitotically active osteoblastoma. From the 5 cases diagnosed as aggressive osteoblastoma in our cohort, 3 showed

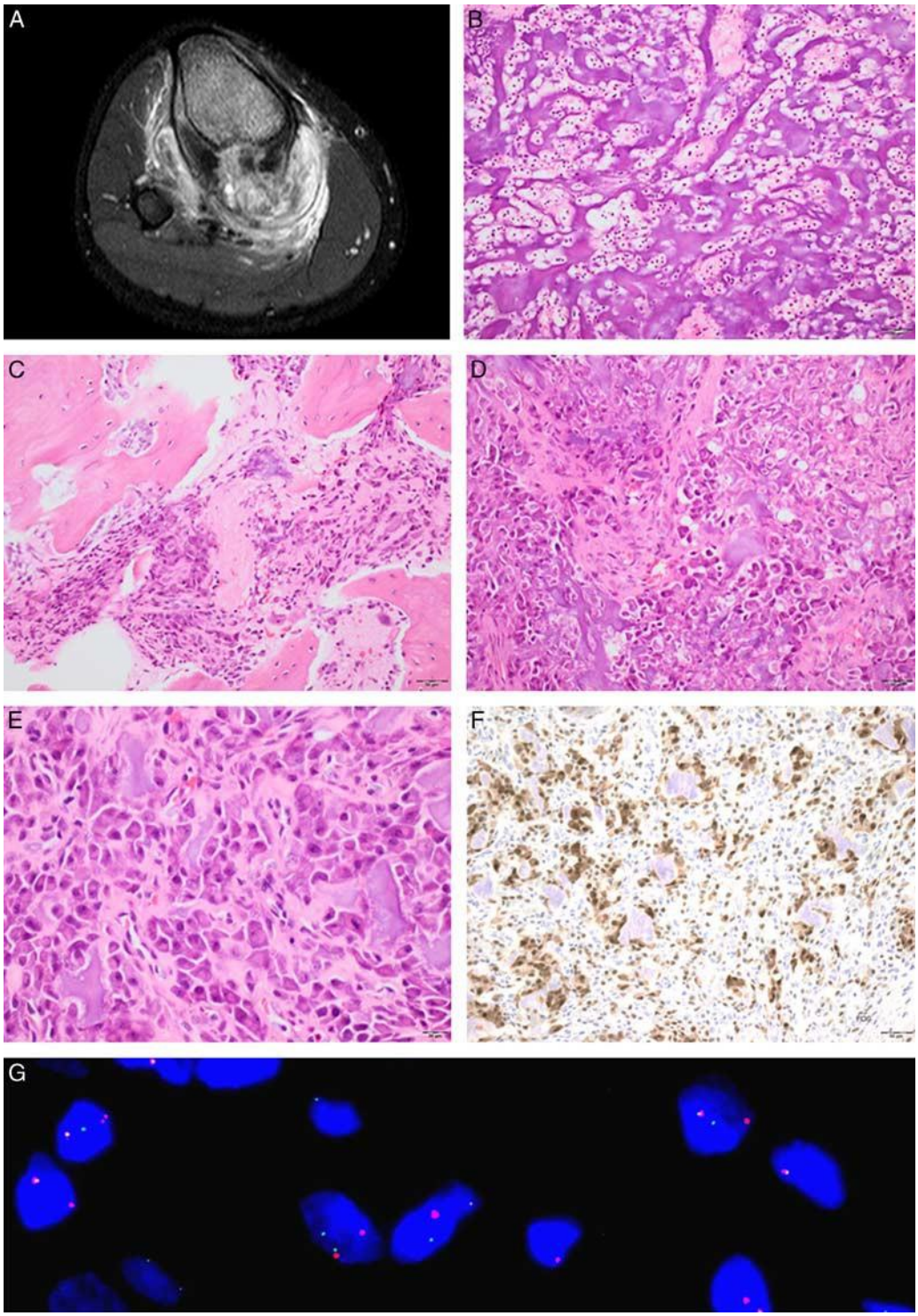

FIGURE 3. Tibial osteoblastoma: (A) axial magnetic resonance image of the right tibia showing focal cortical destruction posteriorly and a large associated hyperintense tumor, with a low signal mineralized margin and perilesional edema. Photomicrographs showing lace-like osteoid deposition (B), tumor growing within the cortical bone (C) and areas with epithelioid morphology (D, E). (F, G) FISH using *FOS* break-apart probes showing clear break-apart signals.

immunoreactivity for c-FOS and 2 were negative. A *FOS* rearrangement was not found in any of the negative cases.

We conclude that c-FOS immunohistochemistry is a helpful ancillary tool in the diagnosis of osteoid osteomas and osteoblastomas, but must be employed with caution in dis-

tinguishing benign from malignant bone-forming tumors. Although c-FOS immunoreactivity may provide more confidence when making a diagnosis of a benign bone-forming tumor, its value is limited due to the expression of the marker being present in a minority of osteosarcomas despite the lack of *FOS* gene rearrangements. The detection of a *FOS* gene rearrangement is a safer means of providing a robust diagnosis in challenging cases.

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