# PERIOSTIN AND OSTERIX EXPRESSION IN ODONTOGENIC AND NON-ODONTOGENIC BONE LESIONS

A Thesis

by

BRYAN GLEN TRUMP

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

| | |
|---|---|
| Chair of Committee, | Yi-Shing Lisa Cheng |
| Co-Chair of Committee, | Jerry Feng |
| Committee Members, | John Wright |
| | Harvey Kessler |
| | Carol Adair |
| Head of Department, | Kathy Svoboda |

May 2014

Major Subject: Biomedical Sciences

# ABSTRACT

Osterix is a transcription factor that is essential for osteoblast differentiation and periostin is a matricellular protein that, in the jaw bones, is expressed in the periosteum and periodontal ligament (PDL). It has been hypothesized that the co-expression of osterix and periostin supports a histogenesis of PDL origin. The purpose of this study was to investigate the expression patterns of periostin and osterix in some jawbone lesions and correlate the results with current concepts regarding the cell of origin of these bone diseases. We also wanted to test whether or not the co-expression of periostin and osterix are indeed PDL- specific. We investigated the expression patterns of periostin and osterix, using immunohistochemistry, in cases of cemento-osseous dysplasia (COD), ossifying fibroma of the jawbones (OFJ), osteoblastoma of the jawbones (OBJ), odontogenic myxoma (OM), osteosarcoma of the jawbones (OSJ), osteosarcoma of the long bones (OSL) and osteoblastoma of the long bones (OBL). All bone lesions (n=57) selected for this study stained positive for periostin. For osterix expression, COD, OSJ, OBL, and most cases of OFJ, OM and OBJ showed positive results. OSL, except for the anaplastic/fibroblastic histological variant, also showed osterix expression. As there is no PDL in the long bones, these results demonstrated that the co-expression of these two proteins was not PDL-specific. The co-expression of osterix and periostin in OM lesional cells suggest a potential differentiation for osteogenesis or PDL origin, despite no calcified material is found in this neoplasm. We also report a novel finding that osterix-positive cells and periostin expression were found

in the lamina propria, and osterix-positive cells were also found in squamous epithelium of normal gingival mucosa.

# ACKNOWLEDGEMENTS

First and foremost, I would like to thank my mentor, Dr. Lisa Cheng, for her unwavering support. She expected the best from me and, in return, gave freely of her time and expertise. Thank you to Dr. Jerry Feng, who let me work in his laboratory and for always being available to answer my questions. I'd also like to thank my other committee members, Dr. John Wright, Dr. Harvey Kessler and Dr. Carol Adair for their input and encouragement. Thanks to Yinshi Ren (Rene) for teaching me the immunohistochemistry protocols and also for the hours spent assisting me.

I want to express my gratitude to my children: Hyrum, Ivy, Delia and Ezra. They are my inspiration. Their smiles, hugs and kisses made each day a good day. Finally, I am eternally thankful to my wonderful wife, Erin, for her patience, understanding, unconditional love and optimism.

TABLE OF CONTENTS

Page

ABSTRACT ..... ii

ACKNOWLEDGEMENTS ..... iv

TABLE OF CONTENTS ..... v

LIST OF FIGURES ..... vi

LIST OF TABLES ..... vii

1. INTRODUCTION ..... 1

2. BACKGROUND AND SIGNIFICANCE ..... 3

3. MATERIALS ..... 9

4. METHODS ..... 11

5. RESULTS ..... 13

6. DISCUSSION ..... 17

7. CONCLUSION ..... 21

REFERENCES ..... 22

APPENDIX ..... 34

LIST OF FIGURES

Page


Figure 1 Representative histology and IHC result for COD…….....34

Figure 2 Representative histology and IHC result for OFJ……...….35

Figure 3 Representative histology and IHC result for OBJ……... 36

Figure 4 Histological features of OBJ that showed no osterix expression…… 37

Figure 5 Representative histology and IHC result for OBL…….. 38

Figure 6 Representative histology and IHC result for OM……39

Figure 7 Representative OM case that showed no osterix expression……...40

Figure 8 Representative histology and IHC result for osteoblastic osteosarcoma of jawbone…….....41

Figure 9 Representative histology and IHC result for chondroblastic osteosarcoma of jawbone…….....42

Figure 10 Representative histology and IHC result for osteoblastic osteosarcoma of longbone……..... 43

Figure 11 Representative histology and IHC result for fibroblastic osteosarcoma of longbone……...44

Figure 12 Representative histology and IHC result for the anaplastic osteosarcoma of longbone……45

Figure 13 Representative histology and IHC result for the giant cell rich osteosarcoma of longbone……46

Figure 14 The expression of periostin and osterix in the epithelium and lamina propria overlying an osteosarcoma of the jawbone……..47

Figure 15 Periostin and osterix expression in a case of fibroma from the buccal mucosa……..49

Figure 16 Periostin and osterix expression in a case of fibroma from the attached gingiva……. 50

LIST OF TABLES

Page

Table 1 Hypothesis of Periostin and Osterix Expression Patterns……. 2

Table 2 Summary of IHC Results for Periostin and Osterix Expression…….. 16

# 1. INTRODUCTION

Osterix is a transcription factor that is essential for osteoblast differentiation (1). Periostin is a matricellular protein that, in the jaw bones, is expressed in the periosteum and periodontal ligament (PDL)(2). Co-expression of osterix and periostin has been found in cementoblasts and in PDL progenitor cells, thereby suggesting a pattern supporting PDL origin (3). The purpose of this project is to investigate the expression patterns of periostin and osterix in some odontogenic and non-odontogenic lesions, and correlate the results with current concepts regarding the histogenesis of these bone diseases. The specific aim is to investigate the expression patterns of osterix and periostin, using immunohistochemistry, in cases of cemento-osseous dysplasia (COD), ossifying fibroma of the jawbones (OFJ), osteoblastoma of the jawbones (OBJ), odontogenic myxoma (OM), osteosarcoma of the jawbones (OSJ), and compare to the results osteosarcoma of the long bones (OSL) and osteoblastoma of the long bones (OBL).

According to the current concepts that odontogenic myxomas are derived from odontogenic ectomesenchyme, that COD and OFJ originate from the periodontal ligament, and OBJ, OBL, OSJ and OSL are derived from osteoblasts, we ***hypothesize*** that the expression patterns will be as seen in Table 1:

Table 1: Hypothesis of Periostin and Osterix Expression Patterns.

| | COD | OFJ | OBJ | OBL | OSJ | OSL | OM |
|---|---|---|---|---|---|---|---|
| Periostin | + | + | - | - | - | - | - |
| Osterix | + | + | + | + | + | + | - |

## 2. BACKGROUND AND SIGNIFICANCE

While bone diseases theoretically can affect any bone in the skeletal system, some bone diseases do appear to be limited to the jaw bones. For decades, pathologists speculated that the lesional cells in those bone diseases unique to the jaw bones most likely originated from cells involved in odontogenesis. No evidence beyond histological features and the unique anatomic location is currently available to support their speculation. The recent findings of osterix and periostin expression during odontogenesis demonstrate that the co-expression of these two proteins is a feature suggesting PDL origin (3). These findings provided a basis to test the speculation regarding the cell origin of those odontogenic diseases.

Benign fibro-osseous lesions (BFOL) are a diverse group of processes that are characterized by replacement of normal bone by fibrous tissue containing a newly formed mineralized product (4). Cemento-osseous dysplasia and ossifying fibroma are two of the most common BFOL that occur in the jaw bones. While cemento-osseous dysplasia is unique to the jaws, ossifying fibroma can also occur in bones from other regions (5, 6).

Cemento-osseous dysplasia can be divided into periapical cemento-osseous dysplasia (PCOD), focal cemento-osseous dysplasia (FCOD) and florid cemento-osseous dysplasia (FLCOD). PCOD and FCOD represent the most common fibro-osseous lesions of the jaws (4, 6-8). All of the cemento-osseous dysplasias occur in tooth-bearing areas. PCOD predominantly involves the periapical region of the anterior

mandible while FCOD exhibits a single site of involvement not in the anterior mandible. FLCOD denotes the most extensive form with multifocal involvement of the jaws not limited to the anterior mandible. PCOD and FLCOD are most commonly found in middle-aged black women, although it may also occur in Caucasians and Asians (9). FCOD typically presents as an asymptomatic, focal, mixed radiolucent/radiopaque lesion with ill-defined borders in the tooth-bearing areas. It was found to occur with greater frequency in women (88%) and in the posterior mandible (77%)(8). Histologic features include a cellular fibrous connective tissue stroma punctuated by irregular osseous and/or cementum-like calcifications. Because cemento-osseous dysplasia is limited to the jaws and arises in close proximity to the periodontal ligament, most investigators believed that these lesions are of PDL origin (9).

Ossifying fibroma is a true neoplasm with a significant growth potential. The most common form of ossifying fibroma occurs in the maxilla and mandible and is typically painless, presenting with expansion of the buccal and lingual corteces where it may expand the inferior aspect of the mandible (10). The radiographic features for OFJ are variable, depending on the amount of calcifications. Usually, they are well circumscribed and often associated with the roots of teeth. Histologically, OFJ is characterized by a variably spindled fibroblastic stroma with varying amounts of mineralized material (bone and/or cementum-like calcifications)(9). In some cases, ossifying fibroma can resemble cemento-osseous dysplasia both radiographically and histopathologically.

As the mineralized material in ossifying fibroma includes bony trabeculae and cementum-like material, it has been suggested that the origin of this neoplasm is odontogenic and of PDL origin. However, neoplasms microscopically identical to an ossifying fibroma have also been reported in long bone and other craniofacial bones such as the orbital, frontal, ethmoid, sphenoid, and temporal bones, leaving these prior theories of origin open to question (6). Therefore, the cell of origin of ossifying fibroma is still unclear. Osteoblastoma is considered a fibro-osseous lesion from osteoblastic origin that is found most often in vertebrae, calvarium, long bones and occasionally in the jaw bones (4).

Odontogenic myxomas (OM) are benign neoplasms that are believed to arise from odontogenic ectomesenchyme, although this is still a topic of debate among pathologists (Neville)(4). Other odontogenic tissues that arise from odontogenic mesenchyme are the dental papilla, follicle, and periodontal ligament. The evidence for odontogenic origin arises from its almost exclusive location in the tooth bearing areas of the jaws, its occasional association with missing or unerupted teeth, and the presence of odontogenic epithelium(11). According to the World Health Organization (WHO), OM is classified as benign tumor of ectomesenchymal origin with or without odontogenic epithelium (12). The odontogenic nature of the myxomas has been challenged by some authors because of its appearance and while consistent with odontogenic ectomesenchyme, it could also represent a more primitive fibroblastic or undifferentiated cell origin (13).

Osteosarcoma of the jaws is a rare entity that accounts for 6-8% of all osteosarcomas (4). The vast majority of head and neck osteosarcomas arise in either the mandible or maxilla (15). Extragnathic osteosarcoma occurs predominantly in the metaphysis of the long bones of adolescents and young adults. It is a malignancy of mesenchymal cells that have the ability to produce osteoid or immature bone. The majority of osteosarcomas demonstrate intramedullary origin. The typical radiographic appearances of osteosarcoma are a destructive mass and immature cloudlike bone formation in the metaphyses of long bones (16). Osetosarcomas of the jaws demonstrate a variety of histologic features but the essential microscopic criterion is the direct production of osteoid by malignant mesenchymal cells.

Osterix, a recently identified zinc finger–containing transcription factor, is expressed in the osteoblasts of all endochondral and membranous bones and is required for osteoblast differentiation and bone formation (1, 17-28). Osterix is required to maintain osteoblast function following adult bone maintenance (29). In humans, two isoforms have been identified and named Sp7-a and -b isoforms, sharing 95% sequence homology with murine Osx (30, 31). In osterix-null mutant mice, endochondral and intramembranous bone formation is arrested, evidence that osteoblast differentiation is prevented (32, 33). Osterix is also important for differentiation into both acellular and cellular cementoblasts (34) as well as in the odontoblasts and PDL cells (3, 35-37). However, its role in bone tumor development is not fully understood. As a transcription factor, osterix exhibits nuclear staining in osteogenic lineage cells, in immunohistochemistry.

Periostin is a matricellular glutamate-containing protein that was originally isolated as an osteoblast-specific factor that functions as a cell adhesion molecule for preosteoblasts and is thought to be involved in osteoblast recruitment, attachment, and spreading (2). Periostin protein is later found to be expressed in a wide variety of normal adult tissues and fetal tissues, such as periosteum (adult and fetal,) skin, PDL, jawbones near developing teeth, placenta, adrenal glands, lung, thyroid, stomach, colon, vagina, ovary, testis, prostate, heart and breast (38-49). Periostin has also been found to be involved in the progression and invasion of certain cancers (50-54), and may be a marker for prediction of malignant behavior in head and neck squamous cell carcinoma and nasopharyngeal carcinoma (64-66). It is considered a potent angiogenic factor and a marker of tumour progression in many types of human cancer (67-69). The protein was renamed "periostin" because of its expression in the periosteum and PDL, indicating a potential role in bone and maintenance of tooth structure (2, 55-57). Occlusal force is thought to be important in the homeostasis of the PDL. These forces might have a role in periostin expression in the PDL and the changes in its expression level during hypofunction may be considered a form of adaptation to environmental changes (58-60). Periostin plays an important role in the determination of bone mass and microstructure in response to loading (61). Periostin is a novel marker for intramembranous ossification, and has been suggested to be a good candidate as a diagnostic tool and/or a therapeutic target in fibrous dysplasia (62). Periostin expression should be found in the extracellular matrix and in the cytoplasm of the cell when using immunohistochemistry.

The purpose of this project is to investigate the expression patterns of periostin and osterix in some odontogenic and non-odontogenic lesions, and correlate the results with current concepts regarding the cell of origin of these bone diseases. While osterix is expressed in both osteoblasts and cementoblasts in the jawbones, the co-expression with periostin (secreted products) would indicate that the cell type is PDL progenitor cells, not osteoblasts. The specific aim is to investigate the expression patterns of osterix and periostin, using immunohistochemistry, in cases of cemento-osseous dysplasia (COD), odontogenic myxoma (OM), ossifying fibroma of the jawbones (OFJ), osteoblastoma of the jawbones (OBJ), osteoblastoma of the longbones (OBL), osteosarcoma of the jawbones (OSJ) and osteosarcoma of the long bones (OSL). The lesional cells of COD and OFJ have been speculated to be of PDL origin, and the neoplastic cells in OFL, OSJ and OSL are believed to show osteoblast differentiation not derived from PDL. Odontogenic myxomas are believed to arise from odontogenic ectomesenchyme and have no osteoid product. The proposal will test the current concepts of histogenesis regarding these bone diseases. The results of this study may provide evidence supporting the speculation of the cell origin in odontogenic and non-odontogenic diseases in the jawbone, and/or provide new diagnostic biomarkers differentiating COD and OFJ from other bone diseases.

# 3. MATERIALS

Formalin-fixed, paraffin embedded human archival specimens and related clinical information (gender, age, duration, location of the lesion, clinical presentation and radiograph, if available) will be retrieved from the database of the Department of Diagnostic Sciences, TAMU-Baylor College of Dentistry. Ten cases of each of the following diagnosis will be selected:

a) COD: Cemento-osseous dysplasia (periapical, focal, or florid)

b) OM: Odontogenic myxoma

c) OFJ: Ossifying fibroma from the jawbone

d) OSJ: Osteosaroma, osteoblastic or chondroblastic types, from the jawbone

e) OBJ: Osteoblastoma from the jawbone

For comparison purposes, ten cases (formalin-fixed, paraffin embedded human archival specimens and related clinical information, as described above) of each of the following diagnosis also will be selected from the database of the Department of Pathology, Baylor University Medical Center (BUMC):

a) OBL: Osteoblastoma of the longbone, without periosteum involvement

b) OSL: Osteosarcoma of the long bone, without periosteum involvement

An established immunohistochemistry protocol, for the two antibodies, will be followed. The following is a list of supplies utilized in the protocol: Xylene (StatLab, Lewisville, Texas), Reagent alcohol (StatLab, Lewisville, Texas),30% Hydrogen

Peroxidase (Fisher Scientific, #704922), phosphate buffered solution (PBS) (Sigma), Bovine Serum Albumin (Sigma, A3059), Goat Serum (Vector, S-1000), Rabbit anti-OSX (Abcam, ab22552), Rabbit anti-periostin (Innovative Research, IR100000)), Biotinylated anti-rabbit IgG (Vector, #BA-1000), ABC Peroxidase Kit (Vector PK-4000), DAB (Sigma, D5637-25G), Trisodium Chloride (Fischer Scientific, EC 201-064-4), Sodium Hydroxide, Citric acid buffer, Sodium citrate buffer, Tween-20 (Fischer Scientific, EC 500-018-3), alarm timer (VWR, Suwanee, GA), latex gloves (VWR, Suwanee, GA), Sterile serolocial pipets (VWR, Suwanee, GA), Accu-mount60 (Baxter, McGaw Park, IL)

# 4. METHODS

Tissue sections (4 micrometers in thickness) will be obtained from the selected cases as described above. The slides will be de-paraffinized with xylene and rehydrated with decreasing concentrations of reagent alcohol followed by phosphate buffered saline (PBS). Antigen retrieval will be done by steaming the slides in 10mM sodium citrate buffer (pH =6.0) for 10 minutes. Endogenous peroxidase activity will be inhibited with 3% hydrogen peroxide buffer (Fischer Scientific, #704922). The slides will then be rinsed with PBS, blocked 3% bovine serum albumin (BSA) and 20% goat serum, followed by incubating with primary antibodies, diluted in 20% goat serum in PBS overnight at 4 °C. The primary antibodies that will be used include OSX rabbit anti-human (Abcam, 1:400) and Periostin rabbit anti-human (Innovative Research, 1:1000) antibodies. The slides will then be washed with PBS and incubated with biotinlylated anti-rabbit IgG (1:200, Vector, #BA-1000) for one hour, at room temperature.. After washing with PBS, antibody detection will be accomplished using ABC peroxidase kit (Vector, PK-4000), following the manufacturers' instructions. The slides will then be incubated with the substrate solution containing diaminobenzidine tetrahydrochloride (DAB, Sigma, D5637-25G) in50 mM trisodium chloride (Fischer Scientific, EC 201-018-3), pH = 7.4. The slides will be counterstained with hematoxylin, dehydrated with ascending concentrations of reagent alcohol followed by three washes of xylene, and then mounted with coverslips with Accu-mount (Baxter, McGaw Park, IL). Sections

incubated with BSA without the primary antibody will be used as the negative control. Sections consisting of periodontal ligament tissue will be used as the positive control.

The expression of osterix and periostin in the specimens will be described based on the observation under the light microscope. Representative images will be photographed. Based on the literature, osterix expression should be found in the nucleus, and periostin expression should be found in the cytoplasm of the cells and in the extracellular matrix.

# 5. RESULTS

The result of periostin and osterix IHC staining is summarized in Table 2 (Page 15). Of the 45 odontogenic bone lesions and 12 non-odontogenic bone lesions (n=57) selected for this study, all stained positive for periostin. However, the staining pattern varied slightly among the various cases. For those lesions with any osteoid or cartilage formation, strong positive staining was found in the matrix adjacent to the lesional cells and less intensity in the connective tissue stroma away from the lesional cells (Figure 1B-10B). In the areas of the lesion where there is minimal matrix formation, the staining was more uniform in its intensity (Figure 11B). It was also apparent that periostin stained the wall of blood vessels (Figure 5B). For odontogenic myxoma, our only lesion without matrix formation, the majority of lesional tissue stained for periostin, with focal areas that did not stain (Figure 6B). The intensity of the staining was uniform for all cases of OM.

For osterix expression, most of the cases selected for this study showed positive results, although some cases of OFJ, myxoma, OBJ, and OSL were negative (See Table 1). All cases of COD expressed osterix, with 70% of the cases showing positive nuclear staining in more than 50% of the lesional cells, and 30% of the cases expressing focal positivity (positive staining in <50% of the lesional cells). The staining was consistently found in the cells adjacent to the calcified material and occasionally found in the cells within the matrix (Figure 1C).

For COF, all but one of the eleven cases were positive (91%) for osterix expression. Positive staining was found in more than 50% of the lesional cells in nine out of 11 cases of COF (82%)(Figure 2C), while one case showed positive staining in less than 50% of the lesional cells. Seventy-five percent of osteoblastoma of the jawbone (n=4) were positive for osterix with one case showing negative result for osterix (Figure 4C). One of the three positive cases had nuclear staining in more than 50% of the lesional cells (Figure 3C) and the other two cases were focally positive (positive staining in <50% of the lesional cells). Both longbone cases of osteoblastoma were positive for osterix (100%) and had nuclear staining in >50% of the lesional cells (Figure 5C).

For OM, 8 of the 10 cases were positive for osterix with 50% of the cases showing positive nuclear staining in more than 50% of the lesional cells, and 30% of the cases expressing focal positivity (positive staining in <50% of the lesional cells). With no osteoid product in the lesional tissue, all nuclear staining was found in the stellate, spindle and round cells within the myxoid stroma (Figure 6C). Two cases did not show any nuclear staining for osterix (Figure 7C).

Cases of osteosarcoma can be sub-classified based on their histological features. A majority of the cases used in our study showed conventional features (osteoblastic osteosarcoma; OSJ Figure 8A, OSL Figure 10A). One case of OSL had a background predominantly composed of multinucleated giant cells (giant cell rich osteosarcoma, Figure 13A) and five cases of OSL were composed primarily of spindle cells with or without anaplastic features (fibroblastic osteosarcoma; Figure 11A and anaplastic

osteosarcoma; Figure 12A). The amount of osteoid found in these five cases was minimal. Of the 10 osteosarcoma of jawbone cases we retrieved from the database of BUMC and BCD, there were 6 cases of osteoblastic osteosarcoma and 4 cases of chondroblastic osteosarcoma. All cases of osteosarcoma of the jawbones were positive for osterix with 70% of the cases showing positive nuclear staining in >50% of the lesional cells, and 30% of the cases expressing focal positivity (positive staining in <50% of the lesional cells).

All osteoblastic and giant cell rich osteosarcomas of the longbone were positive for osterix. Three of the five positive cases (2 osteoblastic, 1 giant cell rich) had positive nuclear staining in >50% of the leisonal cells. No nuclear staining was seen in the giant cells present in the giant cell rich osteosarcoma. The other two cases of the osteoblastic variant were focally positive (<50%). The five cases of anaplastic or fibroblastic osteosarcoma were negative for osterix. Representative photomicrographs of each histological variant of osteosarcoma of the long bone are shown in Figures 10 through 13.

One interesting and unexpected finding was noted in a case of osteosarcoma of the jawbone where the biopsy material included osteosarcoma and the overlying oral mucosal tissue (Figure 14A). Positive staining for periostin and osterix, respectively, were found in focal areas in the neoplasm (Figure 14B and C). However, positive staining for periostin was also seen in the fibrous connective tissue and basement membrane in the superficial lamina propria overlying the neoplasm (Figure 14D and E). Positive nuclear and cytoplasmic staining for osterix was also found in some cells in the

superficial lamina propria and surface squamous epithelium overlying the neoplasm (Figure 14F and G). As periostin expression has not been reported in superficial lamina propria and osterix expression has not been reported in oral mucosal soft tissue, we further investigated their expression in a case of fibroma from the buccal mucosa (Figure 15A) and two cases of fibroma from the gingiva (Figure 16A). Neither periostin (Figure 15B) nor osterix (Figure 15C) was found to be expressed in the normal oral epithelium or laminar propria in the fibroma from buccal mucosa. Periostin (Figure 16B) and osterix (Figure 16C) was expressed in the normal mucosal epithelium and lamina propria in the gingival fibromas.

Table 2**:** Summary of IHC Results for Periostin and Osterix Expression.

| Diagnosis | *n* | Periostin | Osterix |
|---|---|---|---|
| Cemento-osseous dysplasia | 10 | + (100%) | + (100%) |
| Ossifying fibroma of jawbone | 11 | + (100%) | + (91%) |
| Osteoblastoma of jawbone | 4 | + (100%) | + (75%) |
| Osteoblastoma of longbone | 2 | + (100%) | + (100%) |
| Odontogenic myxoma | 10 | + (100%) | + (80%) |
| Osteosarcoma of jawbone | | | |
| Osteoblastic | 6 | + (100%) | + (100%) |
| Chondroblastic | 4 | + (100%) | + (100%) |
| Osteosarcoma of longbone | | | |
| Osteoblastic | 4 | + (100%) | + (100%) |
| Anaplastic/fibroblastic | 5 | + (100%) | - (0%) |
| Giant cell rich | 1 | + (100%) | + (100%) |
| Total | 57 | 57 | 49 |

# 6. DISCUSSION

The purpose of this project was to investigate the expression of periostin and osterix in some odontogenic and non-odontogenic lesions, and correlate the results with current concepts regarding the cell of origin of these bone diseases. We investigated the expression patterns of osterix and periostin, using immunohistochemistry, in cases of COD, OFJ, OM, OBJ, OBL, OSJ, and OSL. As the co-expression of periostin and osterix has been suggested to be a pattern for periodontal ligament (PDL) cell origin (3), we used it as evidence to determine whether the bone diseases selected in this study were of PDL origin or not, and used long bone lesions as a negative control to test whether this determining criterion was true.

Our results showed that all cases of osteoblastoma of long bone and all cases of osteosarcoma of long bone, except the anaplastic/fibroblastic variants, co-expressed periostin and osterix. As there is no PDL in the long bones, these results did not support using the co-expression of these two biomarkers for indicating PDL origin. However, this finding also could be due to the possible periosteum involvement in the biopsies of these longbone lesions. The bony biopsies are always fragmented and there is no way to determine the location of the biopsy specimen relative to periosteum. It was also not clear in the clinical histories of these cases whether or not these long bone biopsies were close to the periosteum. Therefore, we could only conclude that co-expression of periostin and osterix cannot be used to determine the cell of origin of odontogenic and non-odontogenic bone lesions in human biopsy specimens.

No previous study has investigated the IHC expression pattern of periostin and osterix in the bone diseases selected in our study. We found that all of our cases stained positive for periostin. There is a tendency of an increased intensity for periostin at the periphery of osteoid or cartilaginous matrix, especially in cases of COD and OFJ. This finding might be explained by considering that normal periosteum, where periostin expression is found, is located at the periphery of bone and that the neoplastic cells at the periphery of osteoid or calcifications in those bone lesions might be activated to express periostin**.** Our finding that periostin was expressed in all cases of our benign fibro-osseous lesions (COD, OFJ, and OBJ) also appears to be consistent with the finding from a previously published study that periostin was also expressed in fibrous dysplasia (62), another disease in the benign fibro-osseous lesion category.

Osterix was expressed in the majority of the cases included in our study. Osterix was expressed in 75% of OBJ. The two cases of OBL in this study were both positive for periostin and osterix. However, a weakness of this result is the small number of cases. We initially wanted to obtain 10 cases to the results but due to the rare nature of this lesion at our institution, we were unable to obtain this number. Therefore, further investigation will be needed to confirm this preliminary result.

We sub-classified OSL cases into different histological variants, as we found that there was a difference in periostin and osterix expression among different histological variants. The cases in the anaplastic/fibroblastic variant consistently showed negative result for osterix, despite a positive result for periostin. We speculate that this may be related to the dedifferentiation of the neoplastic cells. The anaplastic feature indicates a

wide range of aberrant gene expression in these neoplastic cells and the aberrant pathway appears to include early stage of osteogenesis and the transcription factor, osterix.

With regards to OM, our results did not support our hypothesis, that no periostin or osterix expression would be seen. Interestingly, even in the absence of osteoid, the neoplastic cells in OM still express osterix and periostin. The expression of osterix provided evidence for the potential for osteogenesis although we still cannot determine whether or not the lesional cells may be of PDL origin. Further investigation of SOST expression in OM may be helpful in confirming the osteogenic but not PDL origin of this neoplasm. In our OM cases, all but 2 cases showed positive staining for osterix and we could not find any histological differences in these two cases from the other cases that stained positive for osterix.

An interesting finding was noted in one case of OSJ, where oral mucosa was included in the biopsy specimen, periostin was found in the lamina propria and osterix was found in the squamous epithelium overlying the neoplasm (Figure 14). Both periostin and osterix have not been reported to be expressed in normal oral mucosal fibrous connective tissue or surface epithelium. We didn't know if they would be expressed in normal oral mucosal soft tissue. Therefore, we did additional IHC studies for periostin and osterix in normal mucosal soft tissue involving buccal mucosa and gingiva. We used fibroma cases for this purpose. Fibroma is a reactive oral lesion characterized by a thickening of fibrous connective tissue that forms a lump. The overlying epithelium is normal and the thickening of the connective tissue is believed to

be reactive change to local irritants. Both the epithelium and the fibrous connective tissue are histologically the same as normal oral mucosa.

As we demonstrated that normal gingival mucosa without osteosarcoma expressed both periostin and osterix, we report this novel finding. Further investigation to characterize the periostin-positive cells and osterix-positive cells in normal oral mucosa is needed.

# 7. CONCLUSION

We conclude that:

1) The co-expression of periostin and osterix is not restricted to PDL, and we cannot use this co-expression to test whether the lesional cells are of odontogenic or non-odontogenic origin in human biopsy specimens.

2) Both periostin and osterix are expressed in COD, OFJ, OM, osteoblastoma and osteosarcoma except the anaplastic or fibroblastic variant of osteosarcoma.

3) The expression of osterix in OM indicates a differentiation toward an osteogenic pathway, although the possibility of PDL origin cannot be totally excluded.

4) Osterix-positive cells and periostin expression are found in the lamina propria and squamous epithelium of normal oral mucosa. Further characterization of these osterix-positive cells is needed to determine their nature.

# REFERENCES

1. Cao Y, Zhou Z, de Crombrugghe B, Nakashima K, Guan H, Duan X, et al. Osterix, a transcription factor for osteoblast differentiation, mediates antitumor activity in murine osteosarcoma. Cancer Research. 2005 Feb 15;65(4):1124-8. PubMed PMID: 15734992. Epub 2005/03/01. eng.

2. Horiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, et al. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. Journal of Bone and Mineral Research: the Official Journal of the American Society for Bone and Mineral Research. 1999 Jul;14(7):1239-49. PubMed PMID: 10404027. Epub 1999/07/15. eng.

3. Cao Z, Zhang H, Zhou X, Han X, Ren Y, Gao T, et al. Genetic evidence for the vital function of osterix in cementogenesis. Journal of Bone and Mineral Research: the Official Journal of the American Society for Bone and Mineral Research. 2012 May;27(5):1080-92. PubMed PMID: 22246569. Epub 2012/01/17. eng.

4. Neville B. DD, Allen C., Bouquot J. Oral and Maxillofacial Pathology. St. Louis, Missouri: Saunders; 2009. 968 p.

5. Eversole LR, Sabes WR, Rovin S. Fibrous dysplasia: a nosologic problem in the diagnosis of fibro-osseous lesions of the jaws. Journal of Oral Pathology. 1972;1(5):189-220. PubMed PMID: 4199101. Epub 1972/01/01. eng.

6. Slootweg PJ. Maxillofacial fibro-osseous lesions: classification and differential diagnosis. Seminars in Diagnostic Pathology. 1996 May;13(2):104-12. PubMed PMID: 8734416. Epub 1996/05/01. eng.

7. Waldron CA. Fibro-osseous lesions of the jaws. Journal of Oral and Maxillofacial Surgery: Official Journal of the American Association of Oral and Maxillofacial Surgeons. 1985 Apr;43(4):249-62. PubMed PMID: 3856641. Epub 1985/04/01. eng.

8. Summerlin DJ, Tomich CE. Focal cemento-osseous dysplasia: a clinicopathologic study of 221 cases. Oral Surgery, Oral Medicine, and Oral Pathology. 1994 Nov;78(5):611-20. PubMed PMID: 7838469. Epub 1994/11/01. eng.

9. Wright JM. Reactive, dysplastic and neoplastic conditions of periodontal ligament origin. Periodontology 2000. 1999 Oct;21:7-15. PubMed PMID: 10551173. Epub 1999/11/07. eng.

10. Eversole R, Su L, ElMofty S. Benign fibro-osseous lesions of the craniofacial complex. A review. Head and Neck Pathology. 2008 Sep;2(3):177-202. PubMed PMID: 20614314. Pubmed Central PMCID: PMC2807558. Epub 2008/09/01. eng.

11. Adekeye EO, Avery BS, Edwards MB, Williams HK. Advanced central myxoma of the jaws in Nigeria. clinical features, treatment and pathogenesis. International Journal of Oral Surgery. 1984 Jun;13(3):177-86. PubMed PMID: 6430823.

12. Barnes L EJ, Reichart P, Sidransky D. Pathology and Genetics of Head and Neck Tumors. World Health Organization Classification of Tumors. 2005:316-7. IRAC Press.

13. T. Lombardi CL, J. Samson, and E. W. Odell. S100, α-smooth muscle actin and cytokeratin 19 immunohistochemistry in odontogenic and soft tissue myxomas. Journal of Clinical Pathology. 1995;48(8):759-62.

14. Thiele OC, Freier K, Bacon C, Egerer G, Hofele CM. Interdisciplinary combined treatment of craniofacial osteosarcoma with neoadjuvant and adjuvant chemotherapy and excision of the tumour: a retrospective study. The British Journal of Oral & Maxillofacial Surgery. 2008 Oct;46(7):533-6. PubMed PMID: 18436357. Epub 2008/04/26. eng.

15. Guadagnolo BA, Zagars GK, Raymond AK, Benjamin RS, Sturgis EM. Osteosarcoma of the jaw/craniofacial region: outcomes after multimodality treatment. Cancer. 2009 Jul 15;115(14):3262-70. PubMed PMID: 19382187. Epub 2009/04/22. eng.

16. Yarmish G, Klein MJ, Landa J, Lefkowitz RA, Hwang S. Imaging characteristics of primary osteosarcoma: nonconventional subtypes. Radiographics: a Review Publication of the Radiological Society of North America, Inc. 2010 Oct;30(6):1653-72. PubMed PMID: 21071381. Epub 2010/11/13. eng.

17. Chen F, Zhang X, Sun S, Zara JN, Zou X, Chiu R, et al. NELL-1, an osteoinductive factor, is a direct transcriptional target of osterix. PloS One. 2011;6(9):e24638. PubMed PMID: 21931789. Pubmed Central PMCID: PMC3172249. Epub 2011/09/21. eng.

18. Choi YH, Gu YM, Oh JW, Lee KY. Osterix is regulated by Erk1/2 during osteoblast differentiation. Biochemical and Biophysical Research Communications. 2011 Nov 25;415(3):472-8. PubMed PMID: 22056560. Epub 2011/11/08. eng.

19. Fu H, Doll B, McNelis T, Hollinger JO. Osteoblast differentiation in vitro and in vivo promoted by osterix. Journal of Biomedical Materials Research Part A. 2007 Dec 1;83(3):770-8. PubMed PMID: 17559111. Epub 2007/06/15. eng.

20. Hatta M, Yoshimura Y, Deyama Y, Fukamizu A, Suzuki K. Molecular characterization of the zinc finger transcription factor, osterix. International Journal of Molecular Medicine. 2006 Mar;17(3):425-30. PubMed PMID: 16465388. Epub 2006/02/09. eng.

21. Kaback LA, Soung do Y, Naik A, Smith N, Schwarz EM, O'Keefe RJ, et al. Osterix/Sp7 regulates mesenchymal stem cell mediated endochondral ossification. Journal of Cellular Physiology. 2008 Jan;214(1):173-82. PubMed PMID: 17579353. Epub 2007/06/21. eng.

22. Komori T. [Functions of BMPs, runx2, and osterix in the development of bone and cartilage]. Nihon Rinsho Japanese Journal of Clinical Medicine. 2005 Sep;63(9):1671-7. PubMed PMID: 16164229. Epub 2005/09/17. jpn.

23. Lapunzina P, Aglan M, Temtamy S, Caparros-Martin JA, Valencia M, Leton R, et al. Identification of a frameshift mutation in osterix in a patient with recessive osteogenesis imperfecta. American Journal of Human Genetics. 2010 Jul 9;87(1):110-4. PubMed PMID: 20579626. Pubmed Central PMCID: PMC2896769. Epub 2010/06/29. eng.

24. Matsubara T, Kida K, Yamaguchi A, Hata K, Ichida F, Meguro H, et al. BMP2 regulates osterix through Msx2 and Runx2 during osteoblast differentiation. The Journal of Biological Chemistry. 2008 Oct 24;283(43):29119-25. PubMed PMID: 18703512. Pubmed Central PMCID: PMC2662012. Epub 2008/08/16. eng.

25. Nishimura R, Wakabayashi M, Hata K, Matsubara T, Honma S, Wakisaka S, et al. Osterix regulates calcification and degradation of chondrogenic matrices through matrix metalloproteinase 13 (MMP13) expression in association with transcription factor runx2 during endochondral ossification. The Journal of Biological Chemistry. 2012 Sep 28;287(40):33179-90. PubMed PMID: 22869368. Pubmed Central PMCID: PMC3460424. Epub 2012/08/08. eng.

26. Tang W, Li Y, Osimiri L, Zhang C. Osteoblast-specific transcription factor Osterix (Osx) is an upstream regulator of satb2 during bone formation. The Journal of Biological Chemistry. 2011 Sep 23;286(38):32995-3002. PubMed PMID: 21828043. Pubmed Central PMCID: PMC3190908. Epub 2011/08/11. eng.

27. Tian Y, Xu Y, Fu Q, Dong Y. Osterix is required for sonic hedgehog-induced osteoblastic MC3T3-E1 cell differentiation. Cell Biochemistry and Biophysics. 2012 May 31. PubMed PMID: 22648388. Epub 2012/06/01. Eng.

28. Zhou X, Zhang Z, Feng JQ, Dusevich VM, Sinha K, Zhang H, et al. Multiple functions of osterix are required for bone growth and homeostasis in postnatal mice. Proceedings of the National Academy of Sciences of the United States of America. 2010 Jul 20;107(29):12919-24. PubMed PMID: 20615976. Pubmed Central PMCID: PMC2919908. Epub 2010/07/10. eng.

29. Baek WY, de Crombrugghe B, Kim JE. Postnatally induced inactivation of osterix in osteoblasts results in the reduction of bone formation and maintenance. Bone. 2010 Apr;46(4):920-8. PubMed PMID: 20026264. Epub 2009/12/23. eng.

30. Milona MA, Gough JE, Edgar AJ. Expression of alternatively spliced isoforms of human Sp7 in osteoblast-like cells. BMC Genomics. 2003 Nov 7;4:43. PubMed PMID: 14604442. Pubmed Central PMCID: PMC280673. Epub 2003/11/08. eng.

31. Gao Y, Jheon A, Nourkeyhani H, Kobayashi H, Ganss B. Molecular cloning, structure, expression, and chromosomal localization of the human osterix (SP7) gene. Gene. 2004 Oct 27;341:101-10. PubMed PMID: 15474293. Epub 2004/10/12. eng.

32. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell. 2002 Jan 11;108(1):17-29. PubMed PMID: 11792318. Epub 2002/01/17. eng.

33. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, et al. Targeted disruption of cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell. 1997 May 30;89(5):755-64. PubMed PMID: 9182763. Epub 1997/05/30. eng.

34. Hirata A, Sugahara T, Nakamura H. Localization of runx2, osterix, and osteopontin in tooth root formation in rat molars. The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society. 2009 Apr;57(4):397-403. PubMed PMID: 19124839. Pubmed Central PMCID: PMC2664981. Epub 2009/01/07. eng.

35. Chen S, Gluhak-Heinrich J, Wang YH, Wu YM, Chuang HH, Chen L, et al. Runx2, osx, and dspp in tooth development. Journal of Dental Research. 2009 Oct;88(10):904-9. PubMed PMID: 19783797. Pubmed Central PMCID: PMC3045537. Epub 2009/09/29. eng.

36. Hiraga T, Ninomiya T, Hosoya A, Takahashi M, Nakamura H. Formation of bone-like mineralized matrix by periodontal ligament cells in vivo: a morphological study in rats. Journal of Bone and Mineral Metabolism. 2009;27(2):149-57. PubMed PMID: 19214373. Epub 2009/02/14. eng.

37. Kim JY, Kim BI, Jue SS, Park JH, Shin JW. Localization of osteopontin and osterix in periodontal tissue during orthodontic tooth movement in rats. The Angle Orthodontist. 2012 Jan;82(1):107-14. PubMed PMID: 21806466. Epub 2011/08/03. eng.

38. Dobreva MP, Lhoest L, Pereira PN, Umans L, Chuva de Sousa Lopes SM, Zwijsen A. Periostin as a biomarker of the amniotic membrane. Stem Cells International. 2012;2012:987185. PubMed PMID: 22966238. Pubmed Central PMCID: PMC3395182. Epub 2012/09/12. eng.

39. Elliott CG, Wang J, Guo X, Xu SW, Eastwood M, Guan J, et al. Periostin modulates myofibroblast differentiation during full-thickness cutaneous wound repair. Journal of Cell Science. 2012 Jan 1;125(Pt 1):121-32. PubMed PMID: 22266908. Pubmed Central PMCID: PMC3269025. Epub 2012/01/24. eng.

40. Hamilton DW. Functional role of periostin in development and wound repair: implications for connective tissue disease. Journal of Cell Communication and

Signaling. 2008 Jun;2(1-2):9-17. PubMed PMID: 18642132. Pubmed Central PMCID: PMC2570010. Epub 2008/07/22. eng.

41. Jackson-Boeters L, Wen W, Hamilton DW. Periostin localizes to cells in normal skin, but is associated with the extracellular matrix during wound repair. Journal of Cell Communication and Signaling. 2009 Jun;3(2):125-33. PubMed PMID: 19543815. Pubmed Central PMCID: PMC2721086. Epub 2009/06/23. eng.

42. Norris RA, Damon B, Mironov V, Kasyanov V, Ramamurthi A, Moreno-Rodriguez R, et al. Periostin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. Journal of Cellular Biochemistry. 2007 Jun 1;101(3):695-711. PubMed PMID: 17226767. Pubmed Central PMCID: PMC3393091. Epub 2007/01/18. eng.

43. Merle B, Garnero P. The multiple facets of periostin in bone metabolism. Osteoporosis International: a Journal Established as Result of Cooperation Between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2012 Apr;23(4):1199-212. PubMed PMID: 22310955. Epub 2012/02/09. eng.

44. Morra L, Moch H. Periostin expression and epithelial-mesenchymal transition in cancer: a review and an update. Virchows Archive: an International Journal of Pathology. 2011 Nov;459(5):465-75. PubMed PMID: 21997759. Pubmed Central PMCID: PMC3205268. Epub 2011/10/15. eng.

45. Naik PK, Bozyk PD, Bentley JK, Popova AP, Birch CM, Wilke CA, et al. Periostin promotes fibrosis and predicts progression in patients with idiopathic

pulmonary ibrosis. American Journal of Physiology Lung Cellular and Molecular Physiology. 2012 Oct 5. PubMed PMID: 23043074. Epub 2012/10/09. Eng.

46. Nakamura S, Terashima T, Yoshida T, Iseki S, Takano Y, Ishikawa I, et al. Identification of genes preferentially expressed in periodontal ligament: specific expression of a novel secreted protein, FDC-SP. Biochemical and Biophysical Research Communications. 2005 Dec 16;338(2):1197-203. PubMed PMID: 16259954. Epub 2005/11/02. eng.

47. Wen W, Chau E, Jackson-Boeters L, Elliott C, Daley TD, Hamilton DW. TGF-ss1 and FAK regulate periostin expression in PDL fibroblasts. Journal of Dental Research. 2010 Dec;89(12):1439-43. PubMed PMID: 20940356. Epub 2010/10/14. eng.

48. Shimazaki M, Nakamura K, Kii I, Kashima T, Amizuka N, Li M, et al. Periostin is essential for cardiac healing after acute myocardial infarction. The Journal of Experimental Medicine. 2008 Feb 18;205(2):295-303. PubMed PMID: 18208976. Pubmed Central PMCID: PMC2271007. Epub 2008/01/23. eng.

49. Oka T, Xu J, Kaiser RA, Melendez J, Hambleton M, Sargent MA, et al. Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. Circulation Research. 2007 Aug 3;101(3):313-21. PubMed PMID: 17569887. Pubmed Central PMCID: PMC2680305. Epub 2007/06/16. eng.

50. Ben QW, Jin XL, Liu J, Cai X, Yuan F, Yuan YZ. Periostin, a matrix specific protein, is associated with proliferation and invasion of pancreatic cancer. Oncology Reports. 2011 Mar;25(3):709-16. PubMed PMID: 21225237. Epub 2011/01/13. eng.

51. Kudo A. Periostin in fibrillogenesis for tissue regeneration: periostin actions inside and outside the cell. Cellular and Molecular Life Sciences : CMLS. 2011 Oct;68(19):3201-7. PubMed PMID: 21833583. Pubmed Central PMCID: PMC3173633. Epub 2011/08/13. eng.

52. Kikuchi Y, Kashima TG, Nishiyama T, Shimazu K, Morishita Y, Shimazaki M, et al. Periostin is expressed in pericryptal fibroblasts and cancer-associated fibroblasts in the colon. The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society. 2008 Aug;56(8):753-64. PubMed PMID: 18443362. Pubmed Central PMCID: PMC2443605. Epub 2008/04/30. eng.

53. Kudo Y, Ogawa I, Kitajima S, Kitagawa M, Kawai H, Gaffney PM, et al. Periostin promotes invasion and anchorage-independent growth in the metastatic process of head and neck cancer. Cancer Research. 2006 Jul 15;66(14):6928-35. PubMed PMID: 16849536. Epub 2006/07/20. eng.

54. Sun C, Song C, Ma Z, Xu K, Zhang Y, Jin H, et al. Periostin identified as a potential biomarker of prostate cancer by iTRAQ-proteomics analysis of prostate biopsy. Proteome Science. 2011;9:22. PubMed PMID: 21504578. Pubmed Central PMCID: PMC3100237. Epub 2011/04/21. eng.

55. Kruzynska-Frejtag A, Wang J, Maeda M, Rogers R, Krug E, Hoffman S, et al. Periostin is expressed within the developing teeth at the sites of epithelial-mesenchymal interaction. Developmental Dynamics: an Official Publication of the American Association of Anatomists. 2004 Apr;229(4):857-68. PubMed PMID: 15042709. Epub 2004/03/26. eng.

56. Ma D, Zhang R, Sun Y, Rios HF, Haruyama N, Han X, et al. A novel role of periostin in postnatal tooth formation and mineralization. The Journal of Biological Chemistry. 2011 Feb 11;286(6):4302-9. PubMed PMID: 21131362. Pubmed Central PMCID: PMC3039381. Epub 2010/12/07. eng.

57. Rios H, Koushik SV, Wang H, Wang J, Zhou HM, Lindsley A, et al. Periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. Molecular and Cellular Biology. 2005 Dec;25(24):11131-44. PubMed PMID: 16314533. Pubmed Central PMCID: PMC1316984. Epub 2005/11/30. eng.

58. Afanador E, Yokozeki M, Oba Y, Kitase Y, Takahashi T, Kudo A, et al. Messenger RNA expression of periostin and twist transiently decrease by occlusal hypofunction in mouse periodontal ligament. Archives of Oral Biology. 2005 Dec;50(12):1023-31. PubMed PMID: 15922993. Epub 2005/06/01. eng.

59. Kii I, Nishiyama T, Li M, Matsumoto K, Saito M, Amizuka N, et al. Incorporation of tenascin-C into the extracellular matrix by periostin underlies an extracellular meshwork architecture. The Journal of Biological Chemistry. 2010 Jan 15;285(3):2028-39. PubMed PMID: 19887451. Pubmed Central PMCID: PMC2804360. Epub 2009/11/06. eng.

60. Padial-Molina M, Volk SL, Taut AD, Giannobile WV, Rios HF. Periostin is own-regulated during periodontal inflammation. Journal of Dental Research. 2012 Aug 29. PubMed PMID: 22933606. Epub 2012/08/31. Eng.

61. Bonnet N, Standley KN, Bianchi EN, Stadelmann V, Foti M, Conway SJ, et al. The matricellular protein periostin is required for sost inhibition and the anabolic response to mechanical loading and physical activity. The Journal of Biological Chemistry. 2009 Dec 18;284(51):35939-50. PubMed PMID: 19837663. Pubmed Central PMCID: PMC2791022. Epub 2009/10/20. eng.

62. Kashima TG, Nishiyama T, Shimazu K, Shimazaki M, Kii I, Grigoriadis AE, et al. Periostin, a novel marker of intramembranous ossification, is expressed in fibrous dysplasia and in c-Fos-overexpressing bone lesions. Human Pathology. 2009 Feb;40(2):226-37. PubMed PMID: 18799196. Epub 2008/09/19. eng.

APPENDIX

**Figure 1**. Representative histology and IHC result for COD. A: Histologically, COD is characterized by osteoid or cementum-like calcifications in a fibrous stroma (H&E stain, original magnification at X100). B: Periostin expression was seen throughout the connective tissue stroma with a slight increase in intensity in the fibrous stroma adjacent to the osteoid (original magnification X100). C: Osterix expression was seen in the nuclei of the lesional cells in the fibrous stroma as well as the cells within the calcifications (original magnification X100).

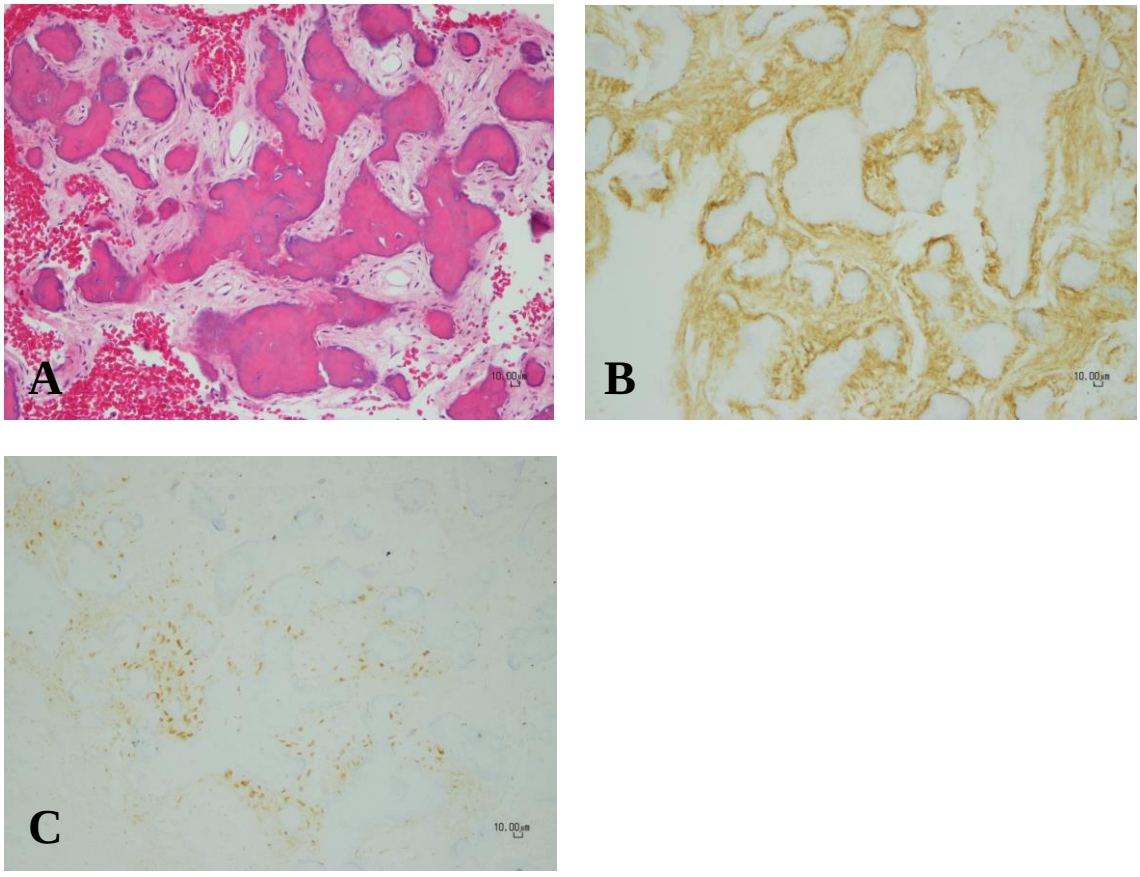

**Figure 2**. Representative histology and IHC result for OFJ. A: Histologically, OFJ consists of fibrous tissue that exhibits varying degrees of cellularity and contains mineralized material (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma with a slight increase in intensity in the fibrous stroma adjacent to the osteoid (original magnification X100). Osterix expression was seen in the nuclei of the cells adjacent to the osteoid as well as in the cells within the calcifications (original magnification X100).

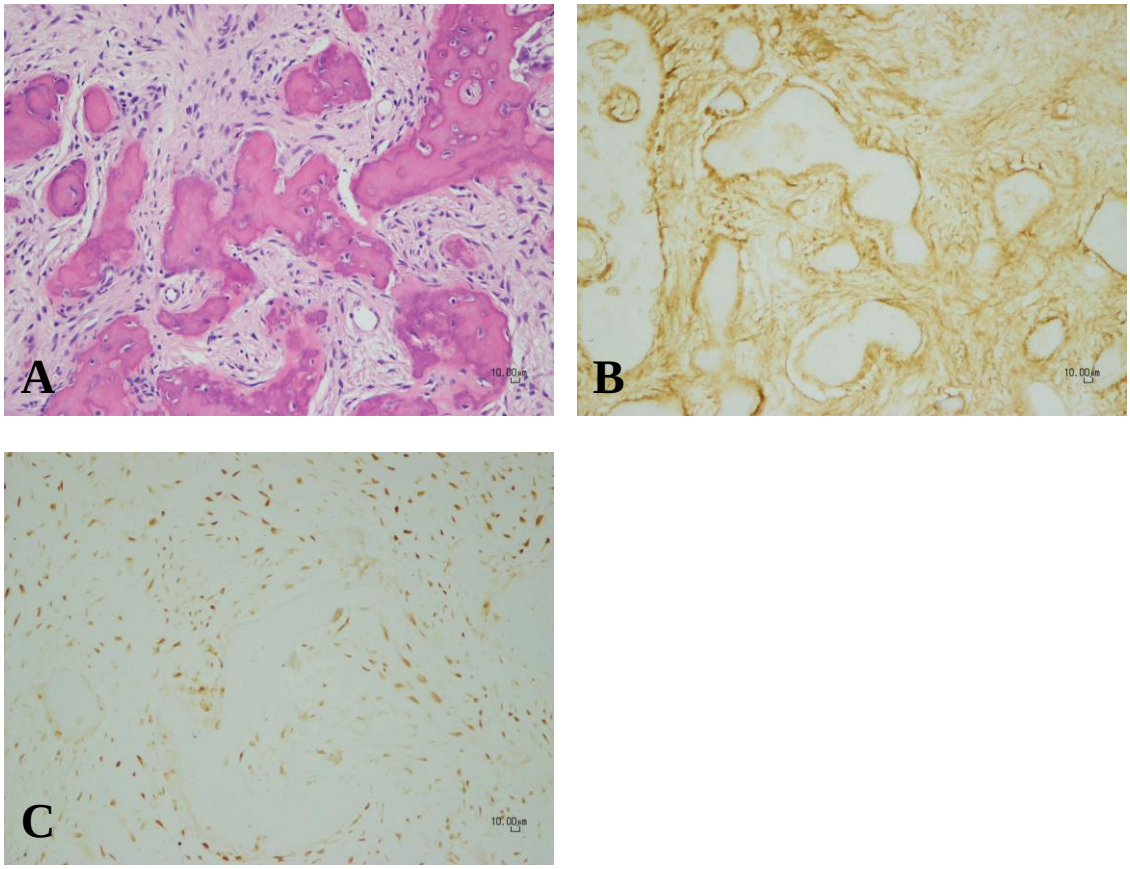

**Figure 3**. Representative histology and IHC result for OBJ. A: Histologically, OBJ is characterized by a mineralized material, with prominent reversal lines, lined by plump osteoblasts (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma with a slight increase in intensity in the fibrous stroma adjacent to the osteoid (original magnification X100). C: Osterix expression was seen in the nuclei of the cells adjacent to the osteoid as well as within the osteoid (original magnification X100).

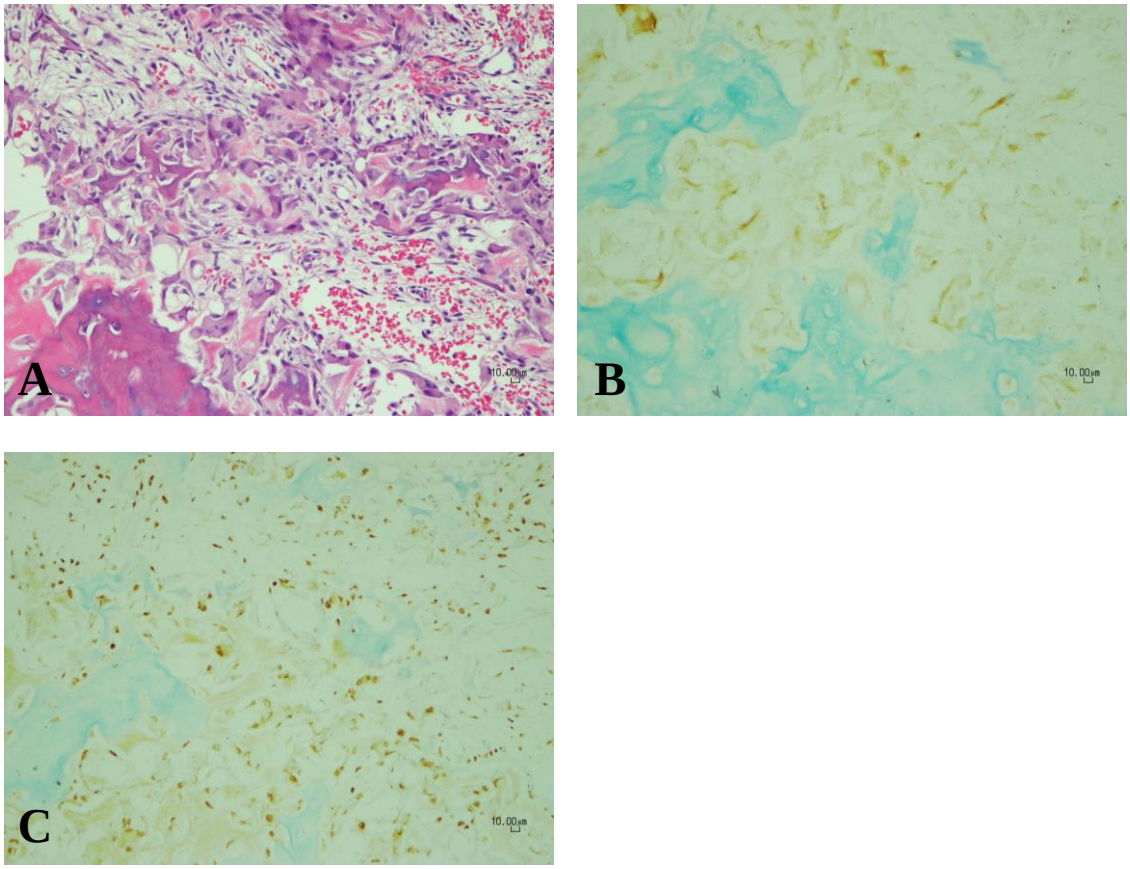

**Figure 4 .** A: Histological features of OBJ that showed no osterix expression (H&E stain, original magnification X100). B: Periostin expression was still seen throughout the connective tissue stroma with a slight increase in intensity in the fibrous stroma adjacent to the osteoid (original magnification X100). C: No osterix expression was found (original magnification X100).

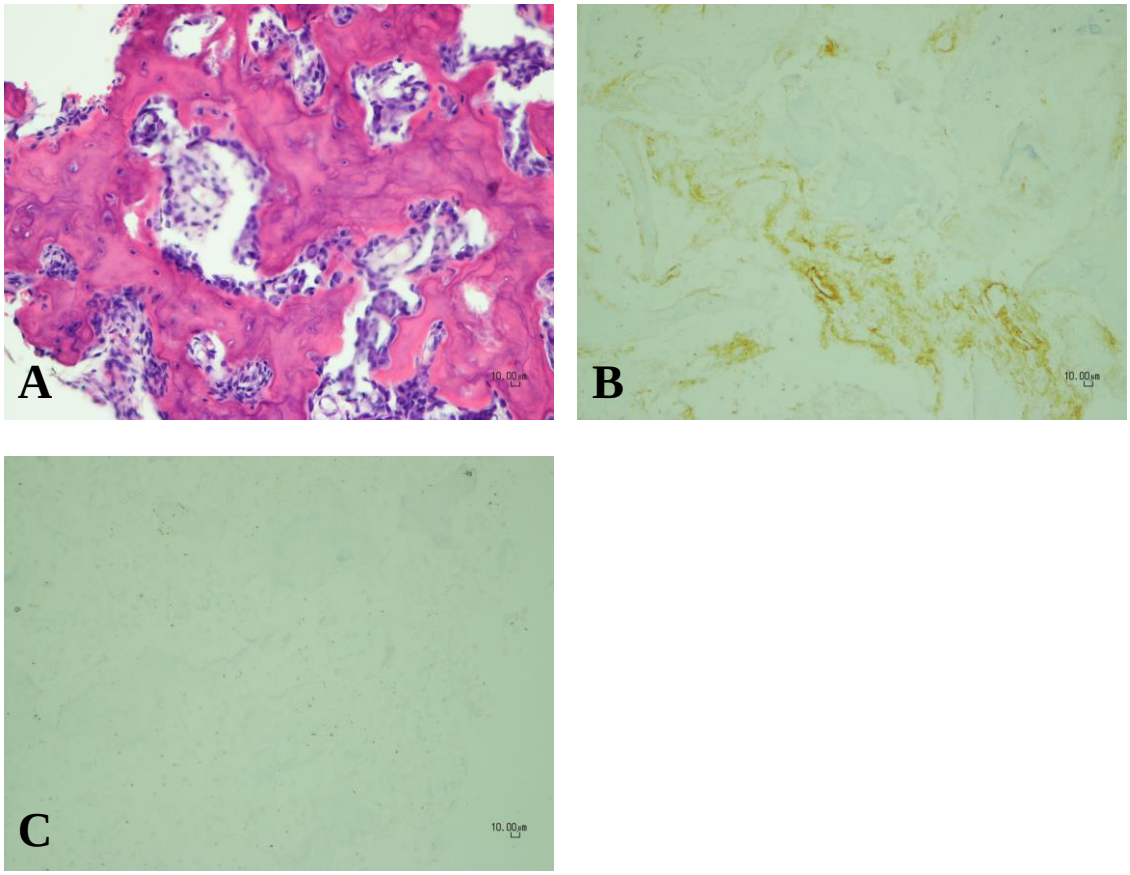

**Figure 5**. Representative histology and IHC result for OBL. A: Histologically, OBJ is characterized by a mineralized material, with prominent reversal lines, lined by numerous osteoblasts (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma with a slight increase in intensity in the fibrous stroma adjacent to the osteoid and in the blood vessel walls (original magnification X100). C: Osterix expression was seen in the nuclei of the cells mostly adjacent to the osteoid and some within the osteoid (original magnification X100).

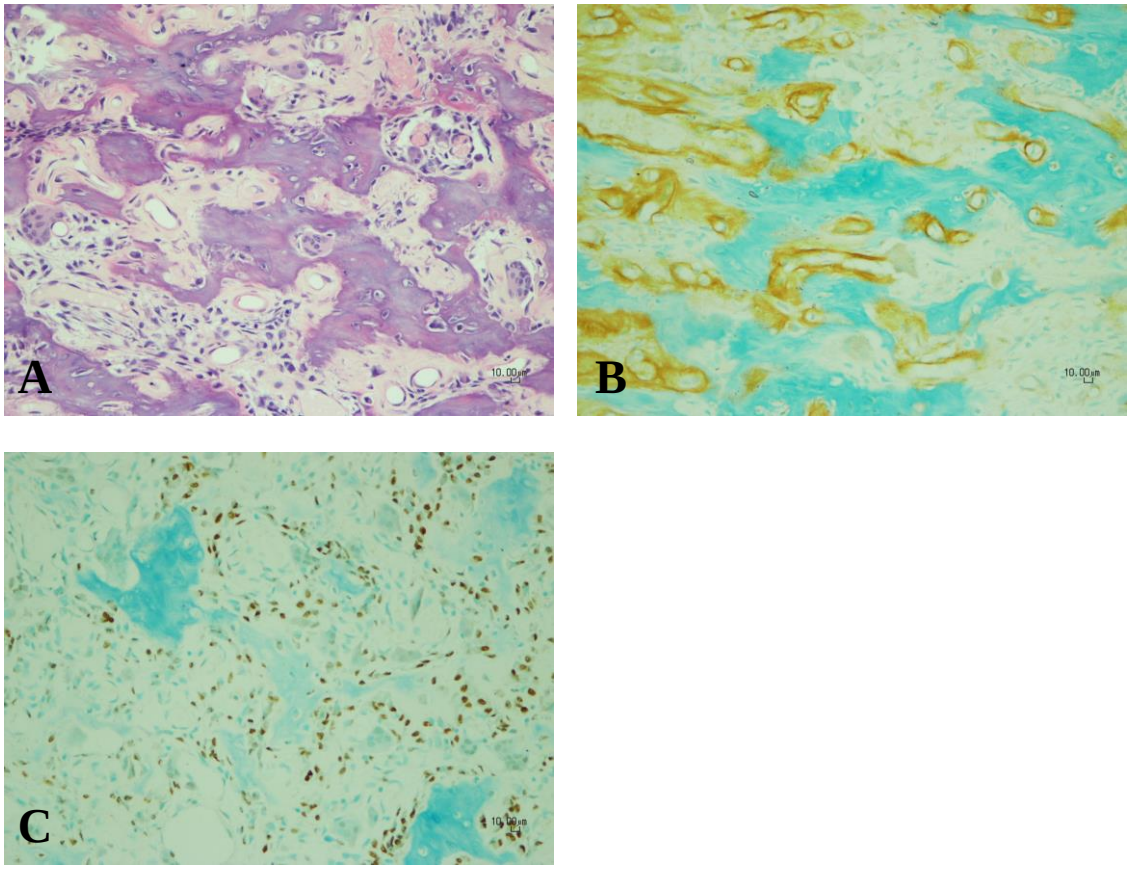

**Figure 6**. Representative histology and IHC result for OM. A: Histologically, OM is characterized by haphazardly arranged stellate, spindle shaped, and round cells in an abundant, loose myxoid stroma (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma (original magnification X100). C: Osterix expression was seen in the nuclei of the lesional cells (original magnification X100).

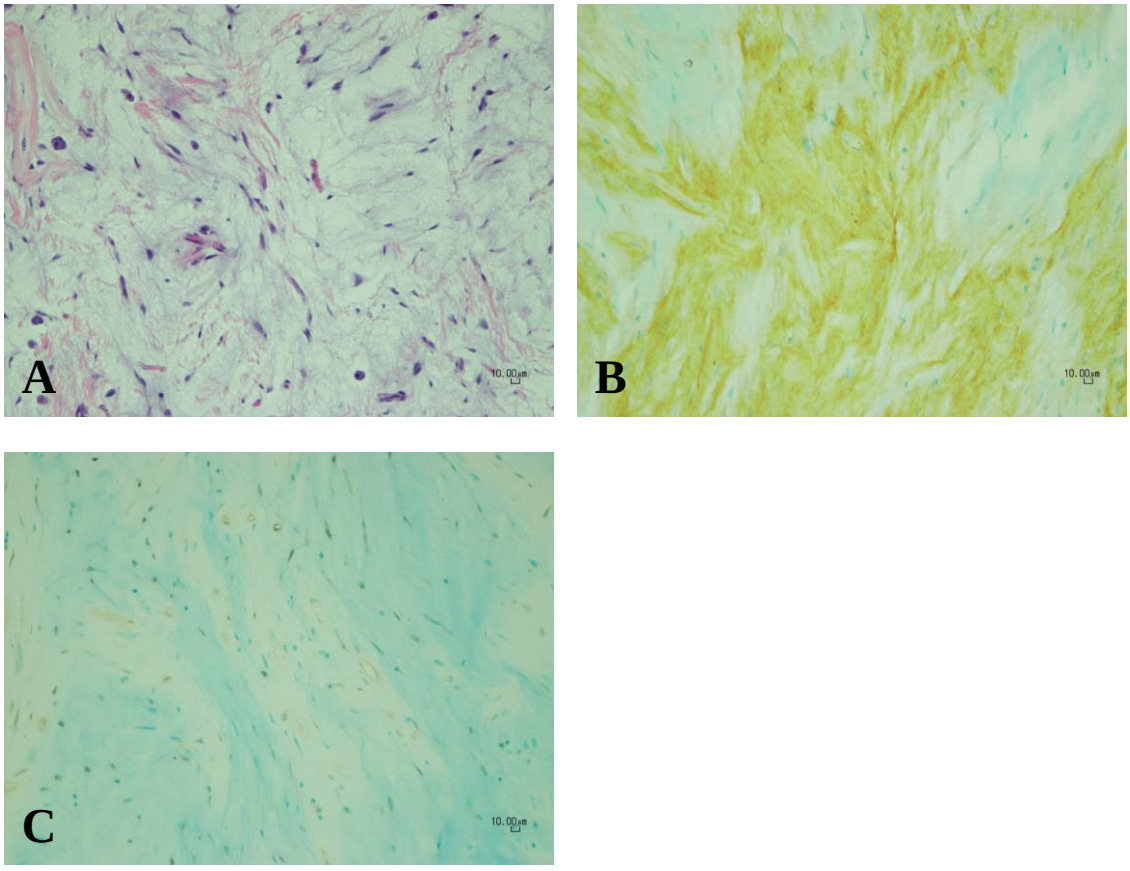

**Figure 7.** Representative OM case that showed no osterix expression. A: Histological features were similar to those seen in other myxoma cases (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma (original magnification X100). C: No osterix expression was found (original magnification X100).

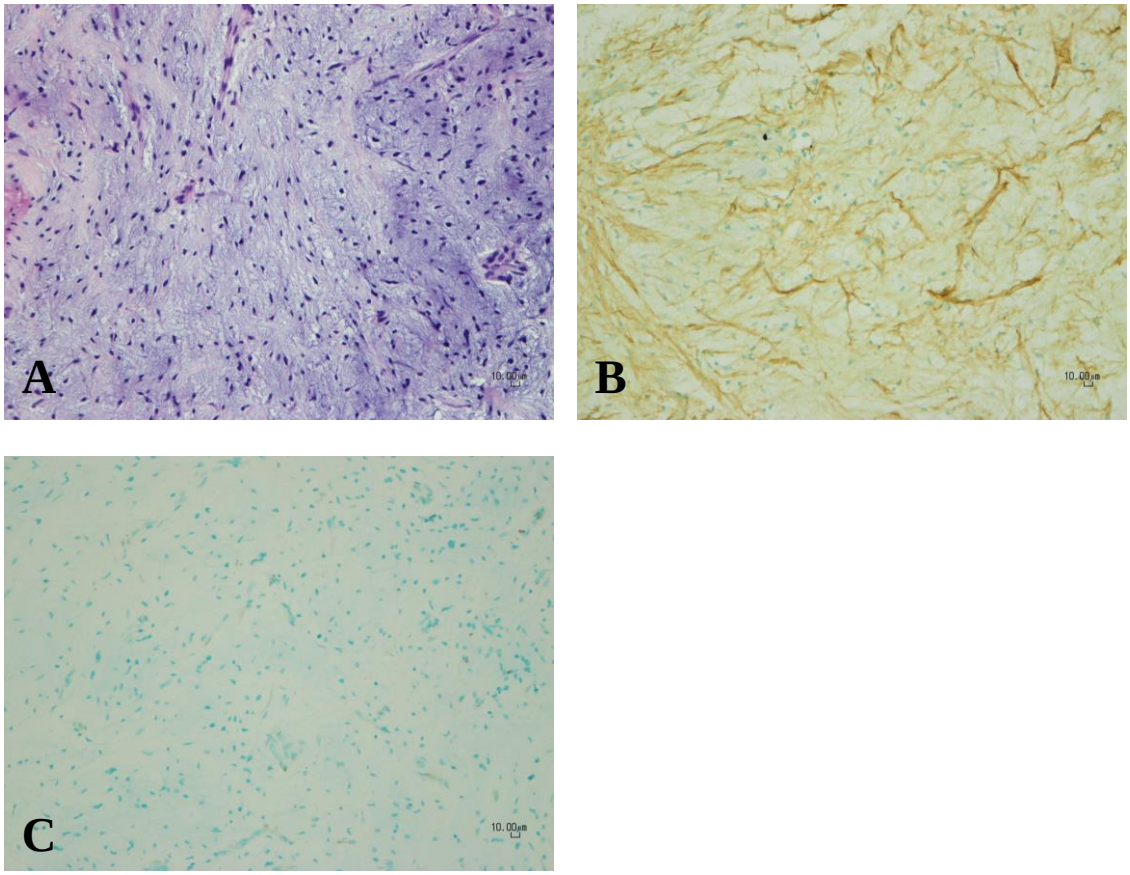

**Figure 8**. Representative histology and IHC result for the osteoblastic osteosarcoma of jawbone (OSJ). A: Histologically, the osteoblastic variant is characterized by the direct production of osteoid by malignant mesenchymal cells ( H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma (original magnification X100). C: Osterix expression was seen in the nuclei of the cells adjacent to the osteoid as well as within the osteoid (original magnification X100).

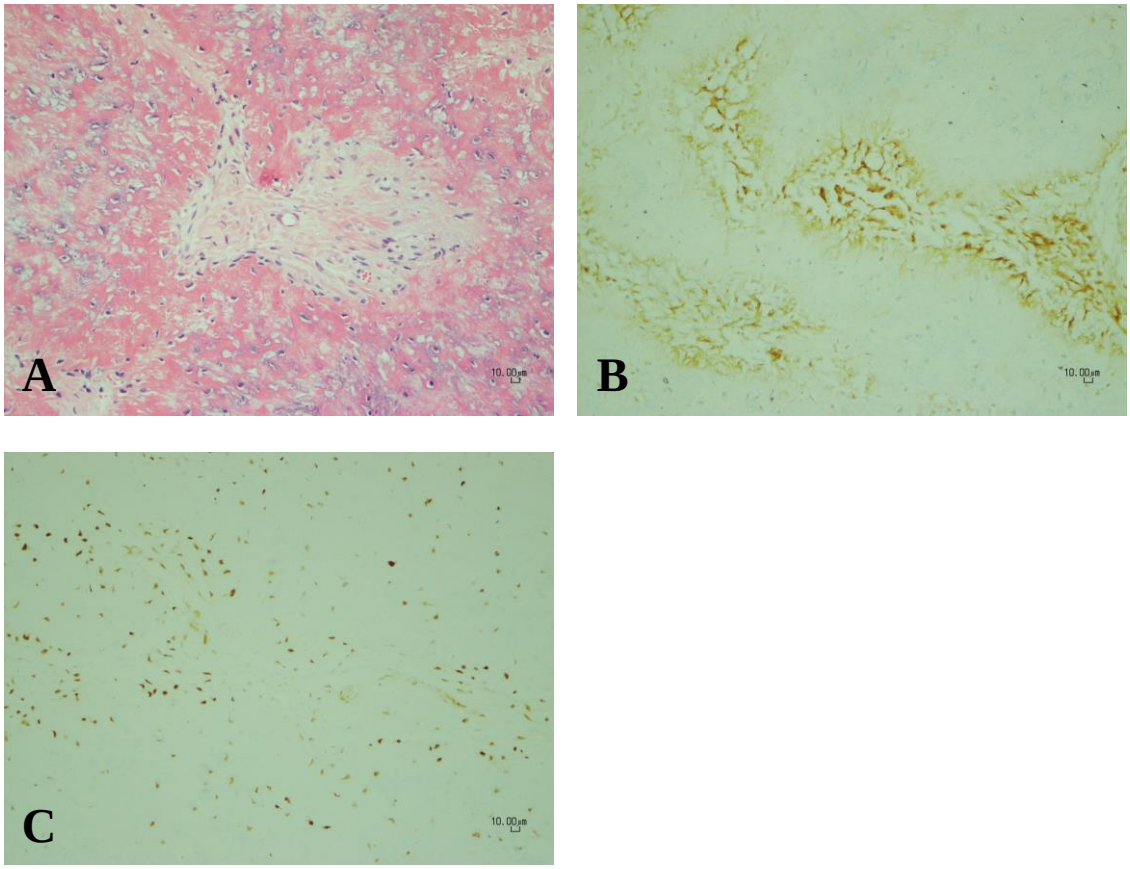

**Figure 9**. Representative histology and IHC result for chondroblastic osteosarcoma of jawbone (OSJ). A: Histologically, the chondroblastic variant is characterized by the direct production of cartilage, as well as osteoid, by malignant mesenchymal cells (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma (original magnification X100). C: Osterix expression was seen in the nuclei of the cells adjacent to the osteoid as well as within the osteoid but not in the immature cartilage (original magnification X100).

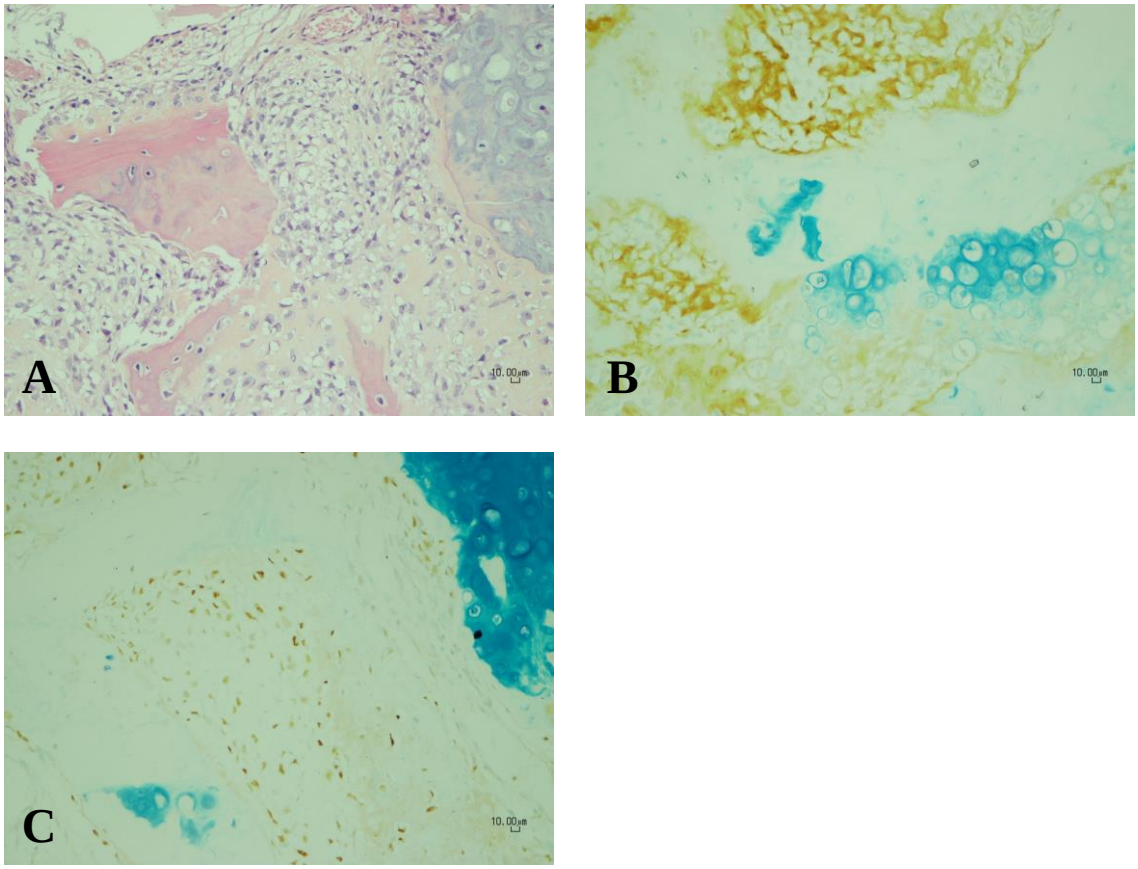

**Figure 10**. Representative histology and IHC result for osteoblastic osteosarcoma of longbone (OSL). A: Histologically, the osteoblastic variant is characterized by the direct production of osteoid by malignant mesenchymal cells (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma (original magnification X100). C: Osterix expression was seen in the nuclei of the cells adjacent to the osteoid as well as within the osteoid (original magnification X100).

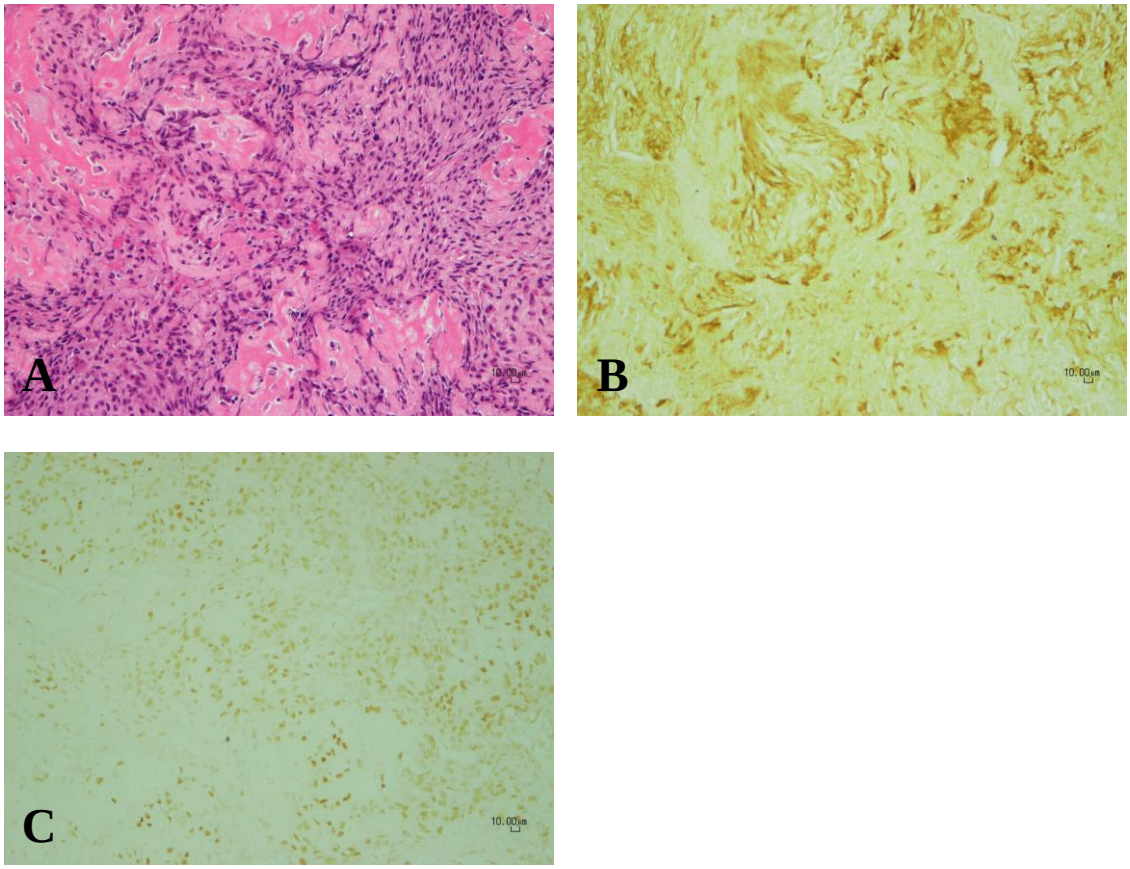

**Figure 11**. Representative histology and IHC result for fibroblastic osteosarcoma of longbone (OSL). A: Histologically, the fibroblastic variant is characterized by malignant mesenchymal spindle cells producing osteoid (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma (original magnification X100). No osterix expression was seen (original magnification X200).

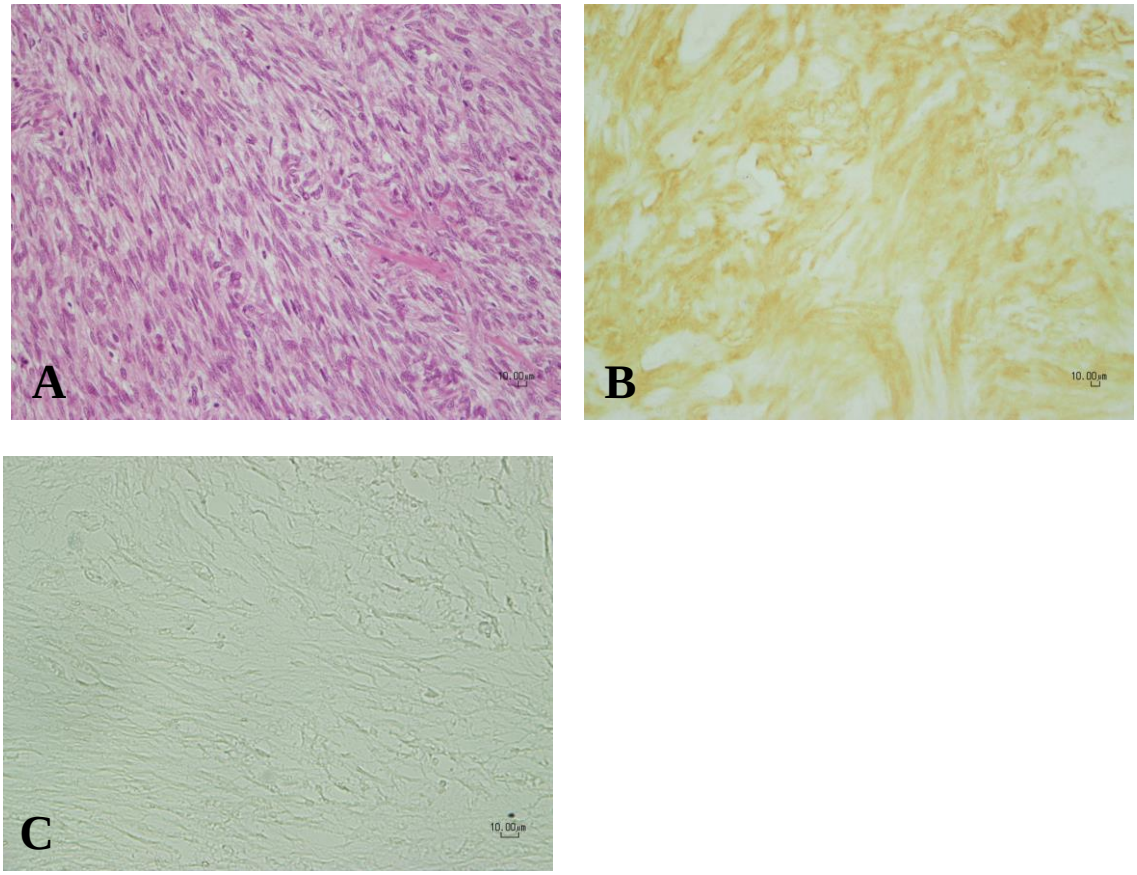

**Figure 12**. Representative histology and IHC result for the anaplastic osteosarcoma of longbone (OSL). A: Histologically, the anaplastic variant is characterized by the minimal production of osteoid by malignant mesenchymal cells that show wide variations in cell size and shape (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma, with variable intensity (original magnification X100). C: No osterix expression was found (original magnification X100).

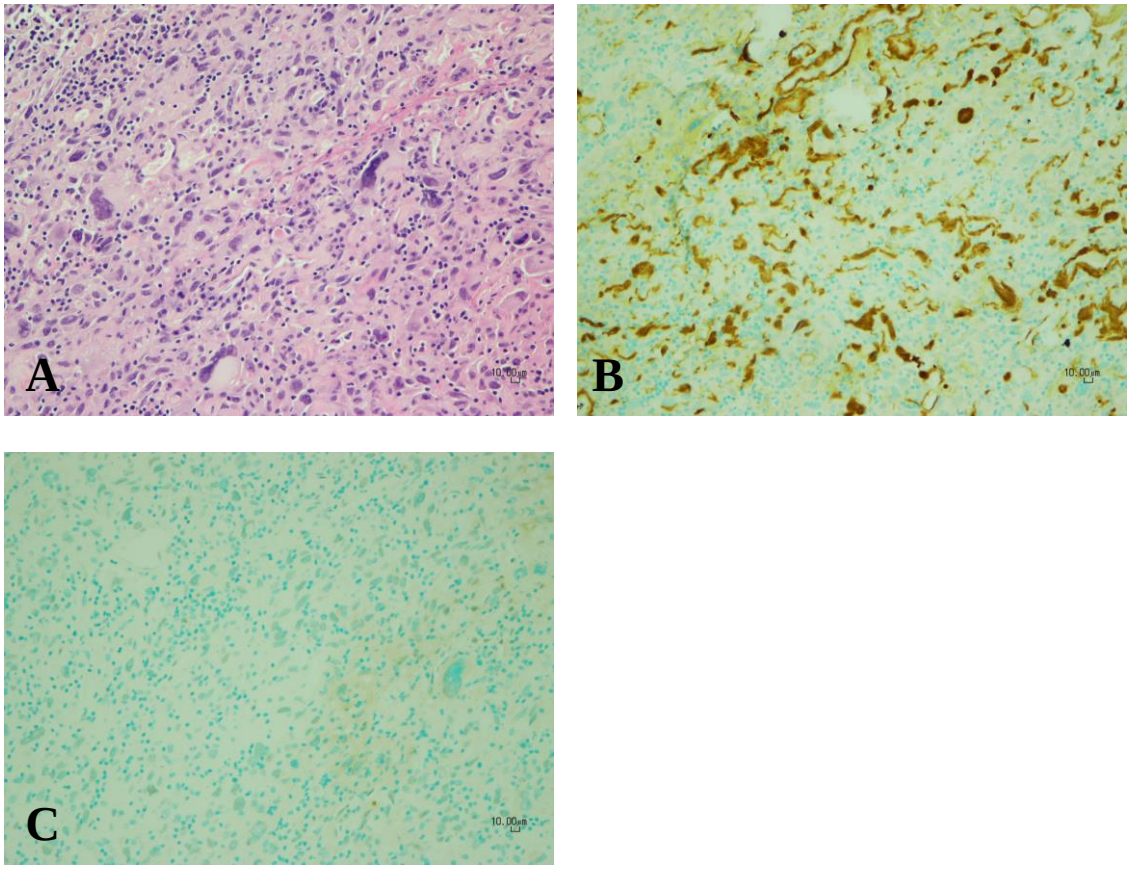

**Figure 13**. Representative histology and IHC result for the giant cell rich osteosarcoma of longbone (OSL). A: Histologically, the giant cell-rich variant is characterized by the production of osteoid by malignant mesenchymal cells with numerous giant cells throughout the lesion (H&E stain, original magnification X100). B: Periostin expression was seen throughout the connective tissue stroma (original magnification X100). C: Osterix expression was seen in the nuclei of the cells adjacent to the osteoid as well as within the osteoid (original magnification X100).

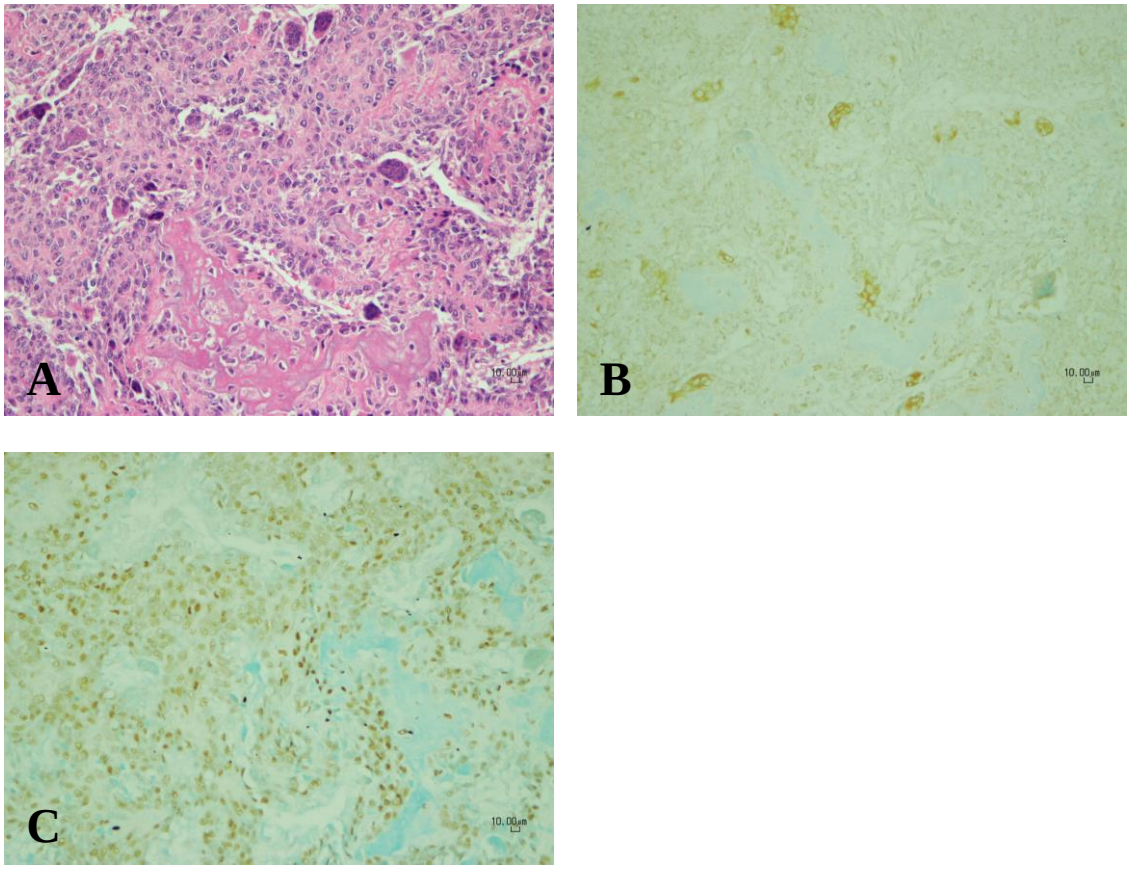

**Figure 14**. The expression of periostin and osterix in the epithelium and lamina propria overlying an osteosarcoma of the jawbone A: The histology of this case showed that the osteosarcoma had eroded the cortical bone and was approaching the oral mucosal surface (H&E stain, original magnification X50). B: Periostin expression was seen in focal areas in the neoplasm (original magnification X100). C: Nuclear staining for osterix was found in the neoplastic cells in some areas (original magnification X100). D and E: Periostin expression was also found in the basement membrane area and superficial lamina propria overlying the neoplasm (D: original magnification X50, E: original magnification X100). F and G: nuclear and/or cytoplasmic staining for osterix was found in cells in the superficial lamina propria and within the surface epithelium (F: original magnification X50, G: original magnification X200).

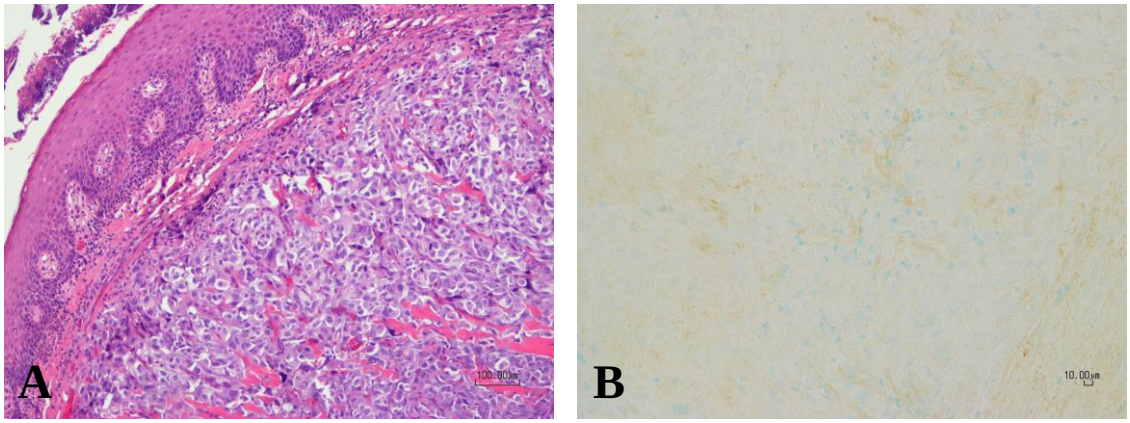

**Figure 14.** Continued.

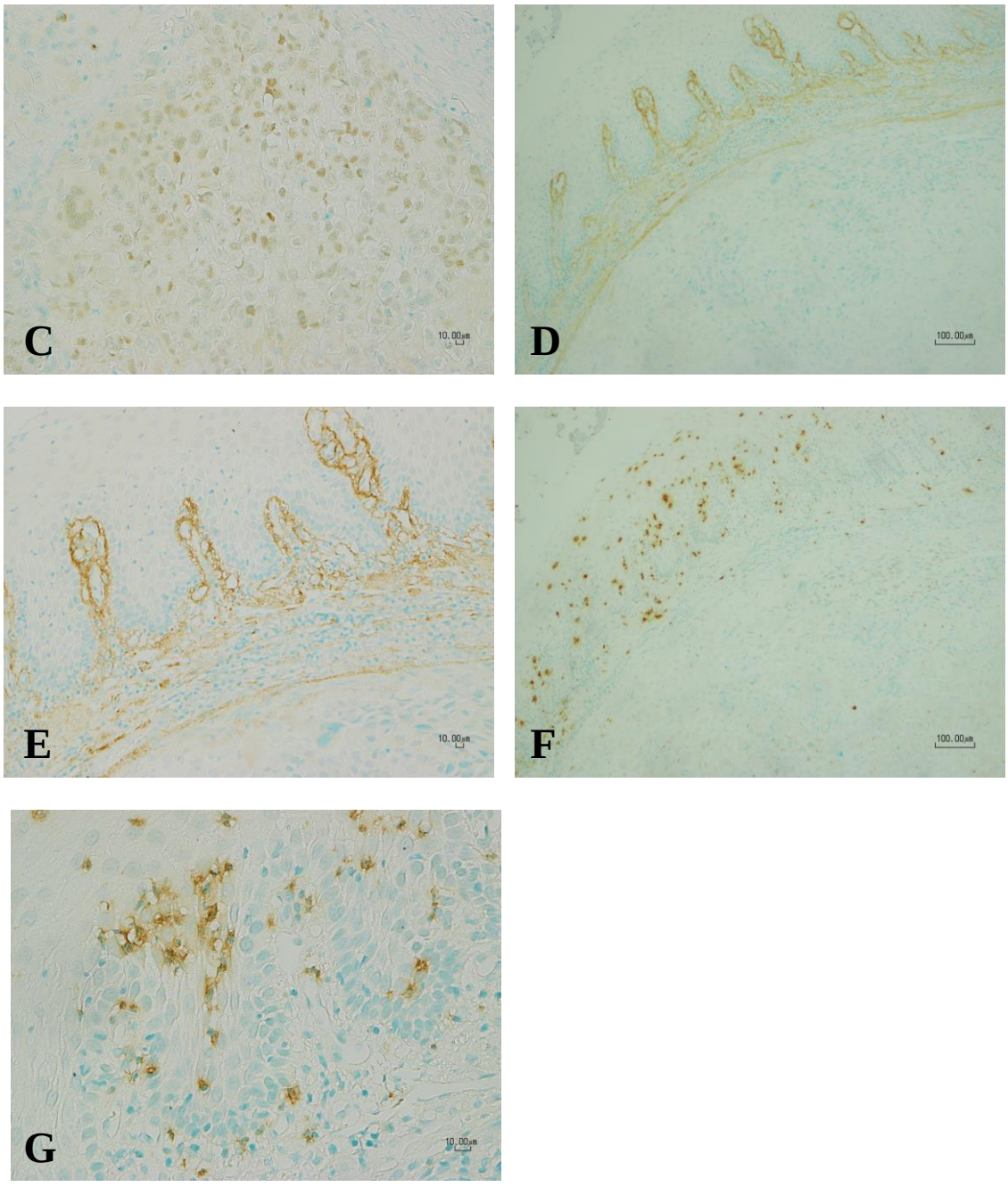

**Figure 15**. Periostin and osterix expression in a case of fibroma from the buccal mucosa. A: This histology of the fibroma showed a thickened fibrous connective tissue in laminar propria and surface squamous epithelium with orthokeratin formation and a normal maturation pattern (H&E stain, original magnification X50) B: No periostin expression was found (original magnification X50). C: No osterix expression was found (original magnification X50).

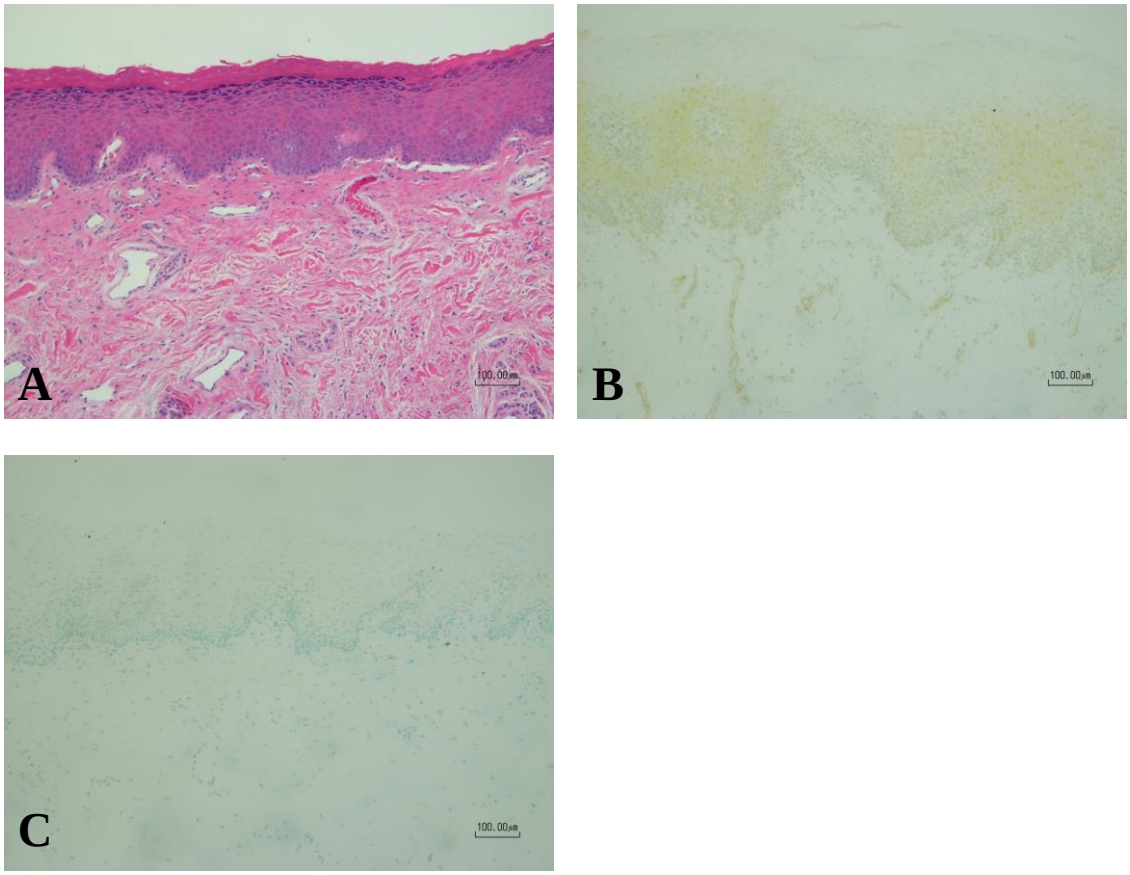

**Figure 16.** Periostin and osterix expression in a case of fibroma from the attached gingiva. A: This histology of the fibroma showed a thickened fibrous connective tissue in laminar propria and surface squamous epithelium with parakeratin formation and a normal maturation pattern (H&E stain, original magnification X50) B: Periostin expression was found in the superficial lamina propria and connective tissue (original magnification X50). C: Osterix expression was found in the surface epithelium and underlying connective tissue (original magnification X50).